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P001

Characterization of adult patients with atopic dermatitis with common filaggrin gene loss of function mutations

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Mutations in the filaggrin gene were first described in *Ichthyosis vulgaris*. These mutations were also identified as a risk factor for atopic dermatitis, especially for a nearly onset and persisting atopic dermatitis as well as associated asthma bronchiale and early sensitizations of the immediate type. So far it is not clear if these mutations are associated with severity or sensitizations to food and aeroallergens in adult patients. We aimed to investigate two common losses of function mutations in the filaggrin gene in a cohort of well characterized adolescent and adult patients with atopic dermatitis regarding disease severity and sensitization levels. We analysed a group of 97 adolescent and adult patients with atopic dermatitis for the two described mutations in the filaggrin gene R501X and 2282del4 by real time PCR. For the mutation R510X we performed a new method not described yet by melting curve analysis on the light cycler, μ instrument and compared patients with or without mutations in disease severity measured by SCORAD, IgE mediated sensitization to aeroallergens and food allergens measured by CAP-FEIA and in a subgroup of 61patients eczematous reactions in the atopy patch test. Mutations in the filaggrin gene were found in 23 out of 97 the patients. 10 patients were heterozygous for R510X, 12 patients were heterozygous for 2282del4, and one patient in each case was homozygous. Disease severity measured by SCORAD did not differ between patients with one of these mutations and patients with the wild type. Both groups did not differ in the frequency of sensitizations to common inhalative allergens. 16 out of 61 patients with results of atopy patch tests showed at least one mutation. In this subgroup the frequency of positive patch test reactions to common inhalative allergens, food allergens, as well as to staphylococcal super-antigens was comparable in patients with and without mutations. Although loss of function mutations in the filaggrin gene clearly are a risk factor for developing atopic dermatitis in childhood, in a cohort of adult patients no difference in disease severity or sensitization levels were seen. Although our cohort of 97 patients is small it points to the fact that common mutations of the FLG gene are no major and strong risk factors for severe AD associated with high sensitization levels in adulthood.

P002

Effects of glycation of the model food allergen ovalbumin on antigen uptake and presentation by human dendritic cells driving the subsequent T cell response

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Advanced glycation end products (AGE) of food proteins resulting from the maillard-reaction after cooking or heating may have particular importance in food allergy. The underlying immunological mechanisms are only poorly understood. The aim of the study was to analyze the effects of AGE derived from the model food allergen-ovalbumin (AGE-OVA) on human dendritic cells (DC), their immunostimulatory capacity and the T cell response compared to regular OVA. For this purpose human monocyte-derived immature DC were exposed to FITC-labeled AGE-OVA and FITC-labeled regular OVA and the uptake was analyzed by flow cytometry and fluorescence microscopy. Furthermore, autologous CD4+ T cell proliferation and cytokine production induced by mature DC loaded with AGE-OVA versus OVA were compared. Finally, expression of RAGE, the receptor for AGE, and activation of the transcription factor NF-κ B by AGE were investigated. Internalization of FITC-AGE-OVA by immature DC was significantly increased compared to FITC-OVA. Blocking the mannose receptor, macropinocytosis or the scavenger receptor strongly reduced uptake of both, FITC-OVA and FITC-AGE-OVA. Comparing CD4+ T cells co-cultured with AGE-OVA versus OVA loaded mature DC; AGE-OVA DC produced more IL-6 and induced a stronger Th2 and weaker Th1cytokine response while there was no difference concerning proliferation of CD4+ T cells. The expression of RAGE was higher on immature DC compared to mature DC. AGE-OVA exposed immature DC showed a stronger expression of RAGE and activation of the transcription factor NF-κ B compared to OVA loaded immature DC. Our data indicate that AGE-OVA may be more immunogenic or allergenic than regular OVA.

P003

F4/80+ antigen presenting cells are not critical for induction of tolerance but for control of contact hypersensitivity

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Specific immune suppression and induction of tolerance are essential processes in the regulation and circumvention of allergies. In the murine model of low zone tolerance (LZT), induced by epicutaneous application of sub-immunogenic doses of contact allergens,, suppressor CD8+ T cells, which inhibit the development of Tc1-mediated contact hypersensitivity (CHS) are generated. Previous experiments excluded skin dendritic cells (epidermal Langerhans cells, dermal dendritic cells) as antigen presenting cells (APC) during induction of LZT, whereas both DC populations are involved in CHS and Tc1 priming as recently reported. In this study, the role of F4/80+ APC in hapten-specific immune responses was analyzed in the models of LZT and CHS. By the use of F4/80-deficient and WT mice, *in vivo* (ear swelling) and *in vitro* (T cell proliferation and cytokine production) parameters were analyzed. In the absence of F4/80+ APC, no alteration of the induction and course of LZT (inhibition of skin inflammation), and the resulting T cell response (generation of CD8+suppressor T cells, abrogation of hapten-specific Tc1 skewing) was observed, excluding a function of F4/80+ APC for hapten-specific tolerance. In contrast, F4/80-deficient mice exhibited a significantly stronger CHS response toward single and repeated epicutaneous sensitizations. The CHS experiments revealed increased skin inflammatory reactions, strong hapten-specific T cell proliferation and augmented Tc1 cytokine productions in the absence of F4/80+ cells as compared to WT animals. These data indicate a regulatory function of F4/80+ APC in CHS as previously demonstrated for F4/80+ myeloid suppressor cells in cancer, infectious and autoimmune models. Thus, our data demonstrate no influence of F4/80+ cells astolerogenic APC on hapten-induced tolerance, but a pivotal function of these myeloid suppressor cells in the control and limitation of allergen-dependent CHS responses.

P004

Differential Regulation of Antimicrobial Peptides in contact dermatitis

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Introduction: Antimicrobial Peptides (AMP) are important effector molecules of the skin to protect the host from surrounding microorganisms. Their expression can be induced by various stimuli and increased expression was reported in psoriasis where bacterial super-infections occur rarely. So far little is known about the role of AMP in contact dermatitis. Therefore, we analyzed the expression and secretion of the most important AMP human beta-defensin (hBD)-2, -3, RNase 7 and psoriasin in experimental contact dermatitis.

Methods: Patients undergoing standardized allergologic epicutaneous testing were included into this study ($n = 32$). Contact dermatitis was induced by sensitization with ≥ one allergen in approximately 50% of these patients, all other patients served as controls. Skin washing fluids were obtained before and 72 h after the contact eczema was induced and in control areas 24 h after occlusion as used for the epicutaneous allergen application. Secreted AMP was measured by established ELISA in the washing fluids. In addition, colony-forming units (CFU) of bacteria within the washing fluid were quantified by plating a standardized amount of washing fluid onto blood agar plates. The expression of AMP was further assessed by immunohistochemical staining in skin biopsies before and 72 h after the contact eczema was induced.

Results: Psoriasin secretion was significantly increased 72 h after the contact eczema was induced in comparison to untreated skin; however, 24 hour occlusion per se induced psoriasin expression at control sites. RNase 7 secretion did not increase after contact eczema was induced. Yet, RNase 7 secretion was decreased24 h under occlusive conditions. Immunohistochemical analyses revealed an up-regulation of psoriasin and hBD-3 expression at sites of contact eczema, whereas RNase 7 and hBD-2 were not induced. The number of bacteria increased significantly 24 h under occlusive conditions and decreased to starting levels when contact eczema occurred. In conclusion, psoriasin and hBD-3 in contrast to RNase 7 and hBD-2 are induced during contact dermatitis. Increased psoriasin expression might be caused by occlusion and not by allergic reaction.

P005

Patients with birch-pollen associated oral allergy syndrome to hazelnut, but notnon-allergic controls, exhibit cross reactive T cell responses between Bet v 1a and Cor a 1.0401

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More than 70% of allergic patients suffering from a primary sensitization to birch pollen develop a secondary food allergy (e.g. to hazelnuts, carrots, apples, celery or others), called oral allergy syndrome (OAS) or the birch pollen-fruit-syndrome. Cross reactivity is mediated by proteins of the pathogen-related protein family 10 (PR-10) homologous to the major birch pollen allergen Bet v 1 (e.g. Cor a 1, Dau c 1, Mal d 1, Api g 1) that cross react with Bet v 1-specific IgE and T cells. In previous reports, itwas demonstrated that *in vitro* generated allergen-specific T cell clones and T cell lines exhibit cross reactivity between Bet v 1a and Cor a 1.0401. In this study, we first analyzed the primary T cell reactivity of patients suffering form OAS ($n = 15$) and non-allergic persons ($n = 8$) in an experimental setting free of exogenous IL-2. We found an allergen-specific CD4+ T cell proliferation to Bet v 1 and Cor a 1 of patients with proven birch pollen allergy and secondary food allergy to hazelnuts (patient history, prick test, CAP and EAST) stimulated by syngenic DC loaded with Bet v 1 or Cor a 1. In contrast, allergen-loaded DC did not induce significant proliferation of T cells obtained from non-allergic individuals. A strong bias to Th2 cytokine secretion (IL-5 and IL-13) was observed in primary cultures of T cells from allergic patients. The induction of Th1 cytokines (IFN-γ) was minimal, IL-10 secretion was not detected. The non-allergic controls exhibited neither allergen-specific Th1- or Th2-cytokine secretion nor IL-10 induction. CD4+ T cells of both groups were re-stimulated with either the identical allergen as in primary stimulations or with the respective cross reactive allergen. In a distinct group of patients, the primary activation of T cells with Bet v 1 or Cor a 1 led to a significant induction of T cell activation in response to the primary or cross allergen. Among the healthy volunteers, no allergen-specific reactivity was detected. Thus, we identified a group of birch pollen allergic patients with T cell cross reactivity to Cor a1.04. Our results emphasize the importance of allergen-specific T cells in the pathogenesis of pollen-associated OAS and they may be the target of novel therapeutic approaches.

P006 (V28)

Evidence that cannabinoid receptor signaling in non-immune cells attenuates allergic contact dermatitis in mice

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G-protein coupled cannabinoid receptors (CB1 and CB2), endogenous lipid ligands (endocannabinoids) and the enzymatic machinery involved in their synthesis and degradation constitute the 'endocannabinoid system' (ECS). Using pharmacological or genetic approaches to block CB receptor signaling, we previously showed that the ECS attenuates allergic contact dermatitis in mice. To investigate the importance of CB receptor signaling in immune cells we performed experiments with wild type and CB receptor-deficient mice involving adoptive transfer of sensitized lymphocytes, bone marrow chimeric mice and sensitization with haptenized dendritic cells. We found that an adoptive transfer of sensitized lymphocytes derived from wild type mice induced significantly stronger allergic inflammation in recipient CB1/2 knockout mice when compared to an adoptive transfer of sensitized lymphocytes derived from CB1/2 knockout mice into recipient wild type mice. Furthermore, CB1/2 knockout mice reconstituted with wild type bone marrow developed stronger allergic inflammation than wild type mice reconstituted with CB1/2 knockout bone marrow. In subsequent experiments we induced allergen-specific immunity by injecting hapten-pulsed dendritic cells derived from wild type mice. Using these standardized conditions for sensitization we confirmed strong allergic inflammation in both CB1/2 knockout and CB1 knockout mice when compared with wild type mice. An adoptive transfer of sensitized lymphocytes derived from wild type mice also induced significantly stronger allergic inflammation in recipient CB1 knockout mice when compared to wild type mice. Taken together, these results provide evidence that CB1-receptor signaling in non-immune cells plays a critical role in the regulation of allergic contact dermatitis. In future experiments we will further address the role of cannbinoid receptor signaling indifferent cell types using tissue specific conditional CB receptor knock out mice.

P007

Determinants of patient satisfaction and quality of life in patients with hand dermatitis. A cross-sectional survey.

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Despite the high prevalence, medical and socio economic importance of hand dermatitis, data on patients' treatment satisfaction and treatment goals are still missing.

We performed a cross-sectional study using a standardized survey, including all patients with chronic hand dermatitis treated between 12/2001 and 11/2008 in the department of dermatology, TU Dresden, Germany. Specific disease-characteristics and aspects of health services research such as treatment satisfaction, treatment goals and quality of life (DLQI) were included. Treatment satisfaction was assessed using a visual analogue scale ranging from 0 (maximum dissatisfaction) to 10 (maximal satisfaction). Determinants of quality of life and patient satisfaction were analyzed by means of regression modeling. 215 patients were included in the study. Mean (SD) treatment satisfaction and DLQI were 6.65 (± 3.06) and 6.28 (± 5.75), respectively. Major determinants of quality of life were the severity of hand dermatitis (modified HECSI-score) ($P < 0.001$), type of hand dermatitis (irritant or allergic contact dermatitis are more incriminating than atopic hand dermatitis ($P < 0.001$)) and the number of relapses ($P < 0.001$). Treatment satisfaction was determined by the number of episodes ($P < 0.001$), patients' self-treatment competency ($P < 0.001$), DLQI ($P = 0.007$), professional competence ($P < 0.001$), physicians' empathy ($P < 0.001$), as well as sufficient information on course, prognosis and treatment options ($P < 0.001$). Gender and occupation had no effect on treatment satisfaction. In summary the quality of life in patients with hand dermatitis is mainly affected by severity, entity and number of relapses. However, due to the findings that besides relapse control major determinants of treatment satisfaction depend on patients' perception of the performance of the physicians as well as on patients' self competence, the daily routine in out patient service should be reassessed and supplemented by educational programs.

P008

Evidence for non-allergic mast cell activation in pollen-associated inflammation

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The occurrence of allergies is rapidly increasing in the western world with allergic rhino conjunctivitis to airborne allergens as the most common symptom. The diagnosis of allergic rhino conjunctivitis and the respective therapy is based on the report of clinical symptoms during pollen season and skin testing with the respective allergen. A positive skin prick test is taken as an indication for IgE-mediated mast cell de-granulation. Since it has recently been shown that pollen not only release allergens but also bioactive lipids which can activate immune cells, we hypothesized that non-allergenic pollen-derived mediators can induce inflammation both in patients as well as in non-allergic mice. First, we assessed levels of specific IgE against birchor ambrosia in patients with seasonal allergic symptoms and positive skin prick test to the allergens. Every 6th patient (24 of 146) with reactivity to birch had no specific IgE against birch, and 35% (13 of 37) of all symptomatic patients with positive history and skin prick test to ambrosia were found to have no specific IgE against ambrosia. To investigate the possibility that non-allergenic pollen-derived mediators can be responsible for mast cell activation, we administered aqueous pollen extracts (APE) from birch pollen or ambrosia i.d. into the ears of naïve mice. This resulted in a rapid, dose-dependent and strong inflammation which was associated with an increased percentage of extensively de-granulated mast cells (MC; 27.2 ± 4.5 in vehicle vs.48.5 ± 4.6 in birch APE injected skin, $P < 0.05$), indicating non-allergic activation of MC by APE from birch. Furthermore, similar injections in MC-deficient KitW/KitW-v mice completely failed to induce inflammation. To confirm that this inflammatory reaction is indeed MC mediated and not due to other deficiencies in the Kit mutant mice was electively repaired the MC-deficiency in the ears of KitW/KitW-v mice by i.d. engraftment with bone marrow-derived cultured MC. MC engraftment completely restored the inflammatory response following injection of APE from birch pollen, thus proving a MC-dependent skin inflammation. Our data show that water soluble, non-allergenic mediators from birch and ambrosia pollen can induce MC-dependent inflammation which may be responsible for seasonal allergy-like clinical symptoms in some patients. Furthermore, these mediators could also serve as adjuvant in sensitization to allergens or aggravate allergic diseases, e.g. in atopic dermatitis.

P009

Influence of acupuncture on type I hypersensitivity itch and the wheal and flare response in adults with atopic eczema - a blinded, randomized, placebo-controlled, crossover trial

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Background: Itch is a major symptom of allergic skin disease. Acupuncture has been shown to exhibit a significant effect on histamine-induced itch in healthy volunteers. We investigated the effect of acupuncture on type I hypersensitivity itch and skin reaction in a double-blind, randomized, placebo-controlled, crossover trial.

Methods: A skin prick-test (house dust mite or grass pollen) was applied to 30 patients with atopic eczema before (direct effect) and after (preventive effect) two experimental approaches or control observation: acupuncture at points Quchi and Xuehai (verum acupuncture (VA), dominant side), 'placebo-point' acupuncture (PA, dominant side), no acupuncture (NA). Itch intensity was recorded on a visual analogue scale. After 10 minutes, wheal and flare size and skin perfusion (via LASER-Doppler) were measured at the stimulus site and the validated eppendorf itch questionnaire (EIQ) was answered.

Results: Mean itch intensity was significantly lower in VA (35.7 ± 6.4) compared to NA (45.9 ± 7.8) and PA (40.4 ± 5.8) regarding the direct effect; and significantly lower in VA (34.3 ± 7.1) and PA (37.8 ± 5.6) compared to NA (44.6 ± 6.2) regarding the preventive effect. In the preventive approach mean wheal and flare size were significantly smaller in VA(0.38 ± 0.12 cm^2 or 8.1 ± 2.0cm^2) compared to PA (0.54 ± 0.13 cm^2 / 13.5 ± 2.8 cm^2) and NA (0.73 ± 0.28 cm^2 or 15.1 ± 4.1 cm^2), and mean perfusion in VA (72.4 ± 10.7) compared to NA (84.1 ± 10.7). Mean EIQ ratings were significantly lower in VA compared to NA and PA in the treatment approach; and significantly lower in VA and PA compared to NA in the preventive approach.

Conclusions: Acupuncture at the correct points showed a significant reduction of type I hypersensitivity itch in patients with atopic eczema. With time the preventive point-specific effect diminished with regard to subjective itch sensation, whereas it increased in suppressing skin prick reactions.

P010

High-resolution transcriptional profiling of chemical-stimulated dendritic cells identifies immunogenic contact allergens, but not prohaptens

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Allergic contact dermatitis is a complex syndrome and knowledge on the *in vitro* detection of small molecular weight compounds, particularly prohaptens, is limited. Therefore we investigated chemical-induced gene expression changes in human antigen presenting cells upon stimulation with immunogenic contact allergens, prohaptens and irritants. Monocyte-derived dendritic cells (moDC) and THP-1 cells were stimulated with the prohapten cinnamic alcohol (Calc), the hapten cinnamic aldehyde (CAld), an irritant and an obligatory sensitizer *in vitro*. Whole-genome screening and consecutive PCR analysis of differential gene expression in moDC stimulated with either CAld or the obligatory sensitizer revealed co-regulation of 11 marker genes which were related to immunological reactions (IL-8, CD1e, CD200R1, PLA2G5, TNFRSF11A), oxidative or metabolic stress responses (AKR1C3, SLC7A11, GCLM) or other processes(DPYLS3, TFPI, TRIM16). In contrast, the prohapten CAlc and the irritant did not change marker gene expression. In THP1-cells, CAld and the positive control elicited similar expression changes in only 4 of the previously identified genes (IL-8, TRIM16, CD200R1 and GCLM). In conclusion we provide important insights into the pathophysiological basis of ACD, identify marker genes suitable for skin hazard assessment and demonstrate that contact-allergenic prohaptens escape *in vitro* detection if their skin metabolism is not taken into account.

P011

Infant eczema is an independent risk factor for mental health problems at age10 years: Results from a prospective birth cohort study (GINIplus)

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Background: Population-based cross-sectional studies suggest an association between eczema and mental health problems in children and adolescents, but the temporal relationship is unclear.

Objective: To assess the association between infant-onset eczema and mental health problems in a prospective study.

Methods: Between 1995 and 1998 a birth cohort study was recruited and followed until age 10 years. Physician-diagnosed eczema, allergic co-morbidities, and a broad set of environmental exposures were assessed at age 1, 2, 3, 4, 6, and 10 years. We investigated the association between infant-onset eczema (age <2 years) and mental health problems at age 10 years according to the strengths and difficulties questionnaire (SDQ) by means of logistic regression modeling (primary research question). Additionally, we analyzed the likelihood of mental health problems at age10 years in relation to the course of eczema in infancy and childhood.

Results: The original cohort included 5 991 newborns, 2 916 of whom were followed until age 10 years and eligible for analysis. Compared to participants never diagnosed as having eczema children with infant-onset eczema had a significantly increased risk for possible/probable mental health problems (SDQ total score) at age10 years (odds ratio (OR) 1.49; 95%-confidence interval (95%CI) 1.13–1.96) and for emotional symptoms (OR 1.62; 95%CI 1.25 –2.09). Eczema limited to infancy predicted a significantly higher risk for conduct problems at age 10. The strength of the association between eczema and emotional problems at age 10 increased with increasing eczema persistence.

Conclusion: Infants with eczema are at increased risk for mental health problems at age 10 years. Even if cleared afterwards, eczema at age <2 years may cause persistent emotional and behavioral difficulties. The underlying biological mechanisms related to eczema in early childhood that may cause behavioral problems and emotional difficulties in subsequent life require further investigation.

P012

Multiple effects of TRAIL on murine mast cells: induction of apoptosis, proliferation and migration

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Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a member of the TNF superfamily of cytokines, has been implicated in immunosuppressive, immunoregulatory and immune effector functions. We aimed to investigate the role of TRAIL in the regulation of various functions in murine mast cells. Primary bone marrow-derived mast cells (BMMC) and the neoplastic mast cell line C57 were found to constitutively express the TRAIL receptor TRAIL-R2. While C57 cells under went apoptosis in response to low concentration of TRAIL, BMMC were resistant to TRAIL-mediated apoptosis at concentrations below 500 ng/ml TRAIL. Stimulation of BMMC with stem cell factor (SCF) or IgE increased the expression of TRAIL-R2 and the susceptibility to TRAIL-mediated apoptosis. Moreover, SCF was found to down-regulate expression of the ligand TRAIL. Interestingly, incubation of BMMC with low concentrations of TRAIL resulted in activation of NF-kappa B signaling associated with increased proliferation and migration, but not with mediator release. To further explore the role of TRAIL in biologic functions of mast cells, we are currently generating mouse mutants with mast cell-specific knockout of TRAIL-R2.Our findings may help to define the complex effects of the TRAIL/TRAIL-R2 system on mast cells and its role in mast cell-driven diseases.

P013

Skin sensory nerves are required for normal sensitization, but not tolerization, to contact allergens

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Allergic contact dermatitis due to contact hypersensitivity (CHS) reactions is one of the major occupational dermatological problems. The induction of low zone tolerance (LZT) may be a promising strategy to prevent harmful allergic reactions to type IV allergens. Nerves have been shown to participate in the pathophysiology of a diverse spectrum of dermatological disorders, including contact dermatitis. Here, we investigated the role of cutaneous sensory nerves in the induction phase of CHS and LZT in a mouse model.

We generated sensory nerve-deficient skin by unilateral surgical denervation of a defined region of back skin in female C57BL/6 mice. Animals sensitized to TNCB on the denervated skin area showed markedly reduced CHS reactions (decreased ear swelling up to 70%, $P < 0.0001$, and hapten-specific T cell proliferation) when compared to sham-operated mice. In contrast, LZT induction was not affected by the absence of skin sensory nerves as CHS responses were significantly diminished by repeated topical low dose application of TNCB on either the denervated or the sham-operated back skin. Our data show that sensory nerves are required for a normal sensitization to contact allergens, allergen-driven skin inflammation and T cell responses, but not for the induction of low dose tolerance. The most likely explanation for the different role of sensory nerves in these two T cell driven responses is that neuropeptides released from cutaneous nerves are involved in the activation of CHS- but not LZT-inducing antigen-presenting cells. The identification of specific neurogenic signals that facilitate sensitization to contact allergens may provide novel targets for the prevention of allergic contact dermatitis.

P014

Specific immunotherapy leads to differential induction of cellular and humoral Bet v 1-specific immune responses in birch pollen allergic individuals

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Correction of an imbalance between allergen-specific T cell subsets and the induction of allergen-specific IgG antibodies are considered to be critical events in promoting allergen tolerance by specific immunotherapy (SIT). In the present study we investigated the impact of SIT on birch pollen allergen (Bet v 1)-specific cellular and humoral profiles in birch pollen allergic patients ($n = 15$) over three years of therapy with emphasis on the induction and maintenance phase as well as during and out of birch pollen season periods compared to immunologic changes in birch pollen allergic subjects treated only symptomatically ($n = 7$) and healthy controls ($n = 8$). Bet v1-specific T helper (Th) 1, Th2, and type 1 regulatory T (Tr1) cell subsets were quantified at decisive time points on the basis of their characteristic cytokine profiles by ELISPOT analysis of peripheral blood mononuclear cells. Additionally, titers of birch pollen allergen-specific IgG4 and IgE antibodies were assessed. Enhanced numbers of Bet v 1-specific, IL-5-producing Th2 cells were found in birch pollen allergic subjects treated only symptomatically and in SIT-treated allergic patients during natural allergen exposure. However, in the latter group Bet v1-specific Th2 cells were only detected in the first birch pollen season after initiation of SIT, but not in the subsequent seasons. Furthermore, only patients on SIT developed an early and prolonged increase of Bet v 1-specific, IL-10-secreting Tr1 cells during the first year of SIT. Interestingly, this increase ceased to appear in the subsequent course of SIT. Unlike both birch pollen allergic cohorts, alterations in the frequencies of Th2 and Tr1 cells could not be detected in healthy individuals. In contrast to the former groups, this cohort was characterized by slightly enhanced numbers of Bet v 1-specific, IFN γ-producing Th1 cells during birch pollen season. While there were no substantial changes of allergen-specific IgE titers during birch pollen season in all three groups, elevated titers of allergen-specific IgG4antibodies continuously increasing over the observation period of three years were detectable in patients receiving SIT, but not in the both control groups. These data show that allergen tolerance induced by SIT is accompanied by a substantial, but transient increase of allergen-specific Tr1 cells during the first year of SIT and augmented IgG4 antibody titers lasting for the entire period of SIT. Thus, both cellular and humoral immunoregulatory mechanisms seem to take share in achieving allergen protection in patients treated by SIT.

P015

Nickel allergic (Ni) patients with complications to Ni containing joint replacement show preferential IL-17 type reactivity to Ni

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Some nickel (Ni) allergic patients develop complications upon Ni-containing arthroplasty. In 15 patients with Ni allergy and 5 healthy controls we assessed lymphocyte reactivity by lymphocyte transformation test (LTT) and questioned potential differences in the *in vitro* cytokine response to Ni in relation to symptom free or complicated arthroplasty. Based on history and patch testing, from 15 Ni allergics (5 without implant, 5 with symptom free arthroplasty and 5 with complicated arthroplasty) and 5 non-allergics LTT was performed using blood mononuclear cells (PBMC). In parallel *in vitro* cytokine response to Ni was assessed by RT-PCR. All 15 Ni allergics showed enhanced LTT reactivity to Ni (NiSO4 10–4 M: mean SI = 8.42 ± 1.8, NiSO4 10–5 M: 4.31 ± 1.13), with a lesser extent in those patients with symptom free arthroplasty (NiSO4 10–4 M mean SI = 3.43 ± 0.52, NiSO4 10-5M: 1.63 ± 0.22). The 5 healthy controls showed no proliferative nor cytokine response (IL-2, IL-4, IL-17, IFN-γ) to Ni. Predominant IFN-γ expression to Ni was found both in the 5 allergics without arthroplasty (NiSO4 10–4 M: 246.48 ± 123.54 fold increase, NiSO4 10–5 M: 36.79 ± 12.42) and less pronounced also in the 5 allergic, symptom free arthroplasty patients. In contrast, in the 5 Ni allergics with arthroplasty-linked complications (pain, swelling, effusion) a predominant, significant IL-17-expression (NiSO4 10–4 M: 62.40 ± 15.35 fold increase, NiSO4 10–5 M: 72.08 ± 27.3) to Ni in combination with reduced IL-4-expression was seen. The preferential IL-17-expression upon Ni exposure in Ni allergic patients with arthroplasty related complications may reflect a susceptibility to develop periimplant allergic hyper-reactivity.

P016

How do dermatologists in Germany approach chronic spontaneous urticaria?

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The EAACI/GA2LEN/EDF/WAO-guidelines for urticaria recommend treating the underlying cause of chronic spontaneous urticaria (csU) when possible. As of yet, there is no information on whether dermatologists in a private practice setting are aware of this recommendation and how often (and with what success) they attempt to identify the underlying cause of disease in their csU patients. Here, we invited all dermatologists in a private practice in Germany to take part in a survey study addressing these questions (CUBA: Chronische Urtikaria – BundesweiteAerztebefragung) and 322 colleagues returned completed surveys. On average, every dermatologist sees 8.5 csU patients per month and one in two dermatologists (51%) claims to be familiar with the current guidelines. 85% of colleagues attempt to identify an underlying cause in their csU patients but only in 24% this attempt is successful. The most common diagnostic procedures performed are laboratory tests (total serum IgE, differential blood count, BSG/CRP), skin prick testing and referrals to ENT specialists and dentists (each performed by >75% of participants). Less often detection of anti-thyroid antibodies, antinuclear antibodies, C1-INH, testing for *Helicobacter pylori* colonization, microbiologic and serologic analyses, and recommendations for a pseudo-allergen-low diet are performed (50–75%). In contrast, only 17.7% routinely use the autologous serum skin test (ASST). These results confirm that csU is a frequent skin disorder. Many but not all colleagues know and follow the current guidelines and try to identify an underlying cause in their csU patients. When they do, skin prick tests and detection of IgE serum levels are preferred over other diagnostic measures, although type-I-allergies are a rare cause of csU. In contrast, less than 1 in 5 dermatologists perform ASSTs to identify auto-reactive csU (one of the most frequent subtypes of csU). Since underlying causes are indentified in less than a quarter of cases, symptomatic treatment remains the mainstay of therapy for the vast majority of csU patients treated in a dermatology private practice setting.

P017

Cost intensive, time consuming and difficult to handle - how dermatologists inGermany view their patients with chronic spontaneous urticaria

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Patients with chronic spontaneous urticaria (csU), one of the most frequent skin diseases, exhibit severely impaired quality of life and a high prevalence of psychiatric co-morbidities. Because of this, the treatment of csU patients by dermatologists is challenging and calls for an understanding of the impact of disease on patients' lives. As of yet, the perception of csU patients by the physicians that treat them has not been studied. To do so, we invited all dermatologists in a private practice setting in Germany to participate in a survey study. 322 completed questionnaires were returned and analysed. We found that the majority of dermatologists perceive the management of csU patients as time consuming (86.3%), cost intensive (69.9%) and in general of low interest from an economical point of view (59.3%). In addition, 18.9% have already experienced reimbursement issues and financial penalties with prescribed therapies, which had changed their therapeutic behavior in most cases (70.5%). CsU patients are seen to have higher expectations (74.2%) and demands (67.7%) and to be more difficult to handle (77.6%) as well as harder to satisfy (53.4%) compared to other dermatologic patient groups. In addition, most participants observed high levels of emotional distress (81.4%) and high rates of mental health problems (63%) in their csU patients. These results demonstrate that dermatologists in Germany find it harder and financially less attractive to care for patients with csU as compared to patients with other conditions.

P018

Changes in disease specific quality of life in chronic urticaria patients can be assessed by CU-Q2oL

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Background: Chronic spontaneous urticaria (CU) is a frequent skin disorder that can severely impact the quality of life (QoL) of affected patients. Recently, we have usedCU-Q2oL, a novel instrument to assess disease specific QoL-impairment in CU-patients, to identify and characterize drivers of QoL-impairment. Here, we have assessed whether CU-Q2oL can also be used to determine changes of QoL associated with changes in disease activity in CU-patients (responsiveness).

Methods: In order to test the responsiveness of the German CU-Q2oL, CU-patients completed the questionnaire at different time points while their treatment was being optimized. In parallel, we assessed disease activity by using the urticaria activity score (UAS) and QoL by using the well-established dermatology life quality index (DLQI), a non-disease-specific QoL-measure.

Results: To date, more than 50 patients completed all questionnaires twice. We found that changes in disease activity are positively correlated with changes of the German CU-Q2oL-scales as well as with the CU-Q2oL total score (global impact). In addition, this correlation seems to be much stronger as compared to the correlation between changes in disease activity and changes of the DLQI-score.

Conclusions: The results demonstrate that the German version of the CU-Q2oL is a valid instrument to detect changes in QoL associated with changes in disease activity and that it is more sensitive in comparison to other, non-disease-specific QoL-measures like the DLQI. Therefore, CU-Q2oL is the instrument of choice to detect treatment effects on QoL in CU-patients in clinical studies as well as in routine patient care.

P019 (V14)

A role for T cell independent interleukin-10 in allergen specific immunotherapy

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Human studies suggest that allergen immunotherapy leads to regulatory immune responses that actively suppress the development of allergic inflammation. Interleukin (IL)-10 productions by allergen-specific T cells has been suggested as one of the main regulatory mechanisms. This was supported by *in vivo* studies in mice in which treatment with IL-10 neutralizing antibody abrogated the beneficial effects of immunotherapy. However IL-10 is secreted by a variety of different cell types and the formal proof that T cell derived IL-10 is required for successful immunotherapy is still lacking. In the present study we address this question using IL-10 null mutants (IL-10–/–) and mice with a cell type specific selective inactivation of the IL-10 gene generated by Cre/loxP-mediated conditional gene targeting. Ovalbumin (ova) sensitized mice were treated with three subcutaneous ova injections on alternate days. One week later mice were challenged by ova inhalation and subsequently allergen specific antibody response, broncho alveolar lavage and airway inflammation was analyzed. In addition, *in vivo* treatment with an IL-10R blocking antibody was used in some of the groups. Subcutaneous immunotherapy was effective in the suppression of allergen induced airway inflammation in wild type mice but not in IL-10 null mutants. Interestingly, immunotherapy was also effective in CD4-Cre+IL-10FL/FL mice with T cell-specific IL-10 deficiency and pretreatment with IL-10R blocking antibody partially reversed this protective effect. Thus allergen immunotherapy is also effective in the absence of T cell derived IL-10 and IL-10 from sources other than T cells contribute to the beneficial effect of subcutaneous immunotherapy. To identify the cellular sources of IL-10 during immunotherapy we made use of novel IL-10 transcriptional reporter mice. IL-10 signals were detectable in different cell subsets of myeloid and lymphoid lineage and varied depending on the organ and time point of analysis. IL-10 signal in cells of the B cell lineage prompted us to analyze the role of B cell derived IL-10 in allergen immunotherapy using mice with a B cell specific IL-10 deficiency (CD19-Cre+ IL-10FL/FL). In the absence of B cell derived IL-10 subcutaneous immunotherapy was still effective in the suppression of allergen induced airway inflammation. In conclusion, our data suggest that neither T cell nor B cell derived IL-10 alone is responsible for the beneficial effect of allergen immunotherapy and that IL-10 from sources other than B and T cells contributes to the beneficial effect of subcutaneous immunotherapy. (S.K. and M.B. contributed equally)

P020

Active transport of contact allergens in human dendritic cells and THP-1 cells is mediated by multidrug resistance related proteins

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The phenomenon of multidrug resistance (MDR) is defined as the ability of a cell to show resistance to a wide variety of structurally and functionally unrelated molecules and is in large part the story of membrane efflux transporters because its principle mechanism is the active transport of substrates out of the cell. The multidrug resistance related proteins (MRPs) function as efflux transporters of a variety of large organic anions or their conjugates in hepatic detoxification, drug distribution, renal clearance and drug resistance of tumor cells. Further we demonstrated that human dendritic cells express a specific pattern of efflux transport proteins especially MRPs. Since their functional role concerning MDR was largely unclear, MRP-mediated efflux activity of human monocyte derived dendritic cells (moDCs) and the monocytic cell line THP-1 was analyzed using an *in vitro* transport assay. Therefore efflux transport of radio labeled contact allergens eugenol and isoeugenol was inhibited using the specific MRP inhibitor indomethacin and the accumulation of substrates in the cells was determined. Treatment with indomethacin (1 mM) decreased the efflux transport of eugenol up to 2.3-fold and of isoeugenol up to 2.1-fold in moDCs. Indomethacin in a concentration of 1 mM strongly reduced the efflux of eugenol in THP-1 cells up to2.5-fold. The efflux of isoeugenol was reduced to a lower extent (up to 1.5-fold). Human DCs have been employed to assess the sensitizing potential of contact allergens and alter their cytokine gene expression profile. In particular marked IL-8 up-regulation has been shown to occur during DC exposure to contact allergens. To survey the functionality of the specific MRP inhibitor indomethacin after stimulation with contact allergens IL-8 regulation was measured by a DC-based *in vitro* assay. Taqman RT PCR analysis of mRNA expression in moDCs cells after treatment with isoeugenol was conducted. Treatment of moDCs with indomethacin after stimulation with isoeugenol resulted in a 12-fold up regulation of IL-8 expression in comparison to control cells without indomethacin treatment. This contact allergen also induced moDC activation indicated by up regulation of IL-8. In addition to previous studies revealing the expression of MRPs in normal human epidermal keratinocytes we are now providing data strongly supporting the functional role of these transport proteins in the active efflux of contact allergens also in antigen presenting cells like moDCs and THP-1 cells.

P021

First results in evaluation of a patch test for fish proteins - a new tool for occupational dermatoses in the fish industry?

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Background: Occupation-related skin diseases, accounting for 25% of work-related illnesses, represent the most common occupational health problem. Hand eczema accounts for 90% of all cases. Previous investigations from the food processing industry have shown that the prevalence of hand eczema is 10–20% among work-related diseases. In particular, the seafood industry has been little investigated. Knowledge about the pathogenesis of hand eczema is incomplete; for the most part there is apparently a cumulative sub-toxic origin. Some fish proteins have a strong allergenic potential. Upon ingestion they can cause an immediate reaction and upon direct skin contact a delayed reaction. Protein contact dermatitis is also a concern. Problems in native fish testing arise from the breakdown of the fish proteins.

Aim: Evaluation of a new epicutaneous test system (fish patch test = FPT) employing lyophilized fish proteins with a mixed study collective.

Results: A mixed collective of patients with psoriasis vulgaris (PV), atopic dermatitis (AD), hand eczema (HE) and healthy controls (Kn) were evaluated at a dermatological rehabilitation clinic of the German pension insurance agency. After 72 h, 33 reactions were observed for the group of 161 test subjects. These were further classified as being 20 cases of questionably positive {?} and 13 cases of simple positive {+} reactions according to the atopic patch test. The group with AD had the most reactions, with 13/46 patients showing 21 reactions. Among the patients with PV, 2/28 showed a total of 8 reactions. All 28 patients with HE had negative results. The 59 Kn individuals showed only 4 questionably positive {?} reactions. The most frequent reactions were determined for the highest concentration of protein (600 g): cod showed the most frequent reactions, followed by salmon, perch/ocean perch (similar results), and only once with herring. The lowest protein levels seldom resulted in reactions, and then only similarly distributed, corresponding to the protein concentration.

Summary: From a total of 2415 test results evaluated, only 33 were positive; thus, we obtained a very low reaction rate of 1.4%. The tendency to produce a reaction rose with the increase in protein concentration, with 2.5-fold more questionably positive reactions than simple positive reactions at the level of 600 µg protein. Thus, there may be a critical threshold for irritative reactions. This first evaluation holds promise for a further practical application of this new FPT on workers in the fishing industry who have intensive contact with fish products and manifest hand eczema.

P022

Proteomic mapping of small chemical-protein interactions at the human skin immune barrier

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Small chemical/allergen-protein interactions are central in chemical-specific human T cell activation during allergic contact dermatitis (ACD). Here we describe a novel immuno proteomic approach to analyze and identify allergen-protein interactions inhuman keratinocytes; cells, which are regarded-together with Langerhans cells as primary allergen contact sites in human epidermis, and which are known to trigger both innate and adaptive immune responses. In this study, a multiplexed proteomic approach was established to identify small-chemical specific target proteins in human skin cells (techniques applied: affinity chromatography, 2-D electrophoresis, Delta2D imaging analysis, mass spectrometry and complementary biochemical protein analytics). More than 40 allergen interacting proteins were identified for most common human contact sensitizer nickel (Ni) and strong contact sensitizer dinitrochlorobenzene (DNCB). Bioinformatics clustering analyses significantly revealed chemical-specific protein pathway associations e.g. of stress induction, as well as apoptosis and metabolism (GO, gene ontology database).

Conclusion: Immunoproteomics and bioinformatics clustering analyses suggest chemical-specific -and potentially cell type specific - allergen-protein interaction processes, most likely being involved in chemical-specific novel T cell epitope generation as well as innate immune responses. This work was supported by the EU: project novel testing strategies for *In vitro* assessment of allergens (sens-it-iv), LSHB-CT-2005 - 018681, www.sens-it-iv.edu.

P023

Identification of a novel mechanism that controls the activity of VEGF protein family members

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VEGF family members are key mediators in vascular remodeling. Structure-function analysis of this protein family is not complete and a detailed analysis is mandatory for a comprehensive understanding of this essential growth factor family in processes such as tissue regeneration and cancer. A common structural feature of VEGF proteins is the alternative expression of a carboxyl-terminal domain of highly basic-amino acids resulting from differential mRNA splicing of a single gene. This heparin-binding domain has been identified as the epitope for the cell surface glycoprotein neuropilin and proteoglycans, both of which are central receptor/binding molecules to control vascular growth. However, it is still unclear how cell functions are specifically modulated by neuropilin or proteoglycan and heparin-binding domain interactions in different VEGF protein members. Furthermore, beside differential mRNA splicing no other mechanism has been identified that controls these interactions. We performed systematic structure-function analysis of different proteins of the VEGF family (Vascular endothelial growth factor-A (VEGF-A), Placenta growth factor (PlGF-1, PlGF-2)). Using screens for protease sensitivity, MALDI-TOF-mass-spectrometry, BIA core analysis and functional *in vitro* and *in vivo* angiogenic assays, we revealed novel functions of the carboxyl-terminal domain of Placenta Growth Factor (PlGF) isoforms. Furthermore, in different VEGF proteins (VEGF-A, PlGF) we identified a specific plasmin-cleavage site, which leads to loss of the heparin binding domain and significant alterations in functional properties of VEGF proteins. Our data strongly support the significant impact of the heparin binding domain of different VEGF proteins on vascular remodeling and emphasize plasmin-mediated cleavage as a common mechanism that controls interactions of different VEGF family members with neuropilins and proteoglycans.

P024

The dermato-endocrine regulator melatonin modifies the time- and dose-dependent UV-response in a human full skin model *in vitro*

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Based on extensive investigation of melatonin in human cell models (keratinocytes, fibroblasts and melanocytes), a great variety of melatonin actions, mechanisms, intracellular localized synthesis and metabolism as well as receptor expression has been unraveled, identifying melatonin as a key player in cutaneous biology and dermato-endocrinology. In recent years, melatonin was identified as a strong antioxidant and cell survival protectant through differentially regulated anti-apoptotic effects in UV-induced damage in human keratinocytes. Here, we used a human full skin model to characterize the dose dependent UV-response related to pigmentation and UV-related stress events. Human full thickness skin was irradiated with UVB at doses of 50, 100 and 300 mJ/cm^2 and frozen directly, 24 and 48 h after UV-irradiation. HE-staining for sunburn-cells, Masson-Fontana (MF) staining, Ki67/TUNEL-assay and casp-3 and p53 immuno fluorescence was performed for every UV-dose and time dependent condition. It was shown, that sunburn cells were detected as early as 24 h after UV-exposure, followed by a UV-dose dependent increase up to 300 mJ/cm^2. Pigmentation (MF) showed an immediate UV-dose dependent decrease followed by a reactive increase with increasing UV-doses at 24h. Ki-67 decreased and vice versa apoptotic fragmented DNA (TUNEL) increased.Casp-3 detection was detected as early as 24 h post irradiation (300mJ/cm^2) with further increase at 48 h post irradiation, while p53 showed maximum activation at24 h in a UV-dose dependent manner. Pretreatment with melatonin (10–3 M) for 1hr before UV-irradiation significantly prevented sunburn-cell formation ($P < 0.001$) and reduced casp-3 as well as p53 activation ($P < 0.001$). Thus, the dermato-endocrine regulator melatonin showed significant protection against UV-induced damage mainly by reducing apoptosis-related events.

P025

Identification of somatostatin as a negative regulator of epidermal wound healing

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The peptide hormone somatostatin (SST) and its five G protein-coupled receptors (SSTR1-5) were described to be present in the skin, but their cutaneous function(s) are unknown. By using receptor specific agonists we show here that the SSTRs expressed in keratinocytes are functionally coupled to the inhibition of adenylatecyclase. Treatment with SSTR4 and SSTR5/1 specific agonists significantly influences the MAP kinase signaling pathway. As epidermal hormone receptors are known to regulate re-epithelialization following skin injury, we investigated the effect of SST on proliferation and migration of human keratinocytes. Our results show that SST leads to a significant inhibition of cell migration and proliferation. Migrating keratinocytes treated with SST show altered cytoskeleton dynamics with delayed lamellipodia formation. Furthermore, the activity of the small GTPase Rac1 is diminished, providing evidence for the control of the actin cytoskeleton by somatostatin receptors in keratinocytes. While activation of all receptors leads to redundant effects on cell migration, only treatment with a SSTR5/1 specific agonist resulted in decreased proliferation. Consistent with the impaired proliferation and migration we observe delayed re-epithelialization in an *ex vivo* wound healing model. Thus, SST is a negative regulator of epidermal wound healing.

P026 (V31)

Angiotensin AT2-receptor stimulation modulates proliferation and differentiation in primary human keratinocytes: a novel pharmacological concept for the treatment of psoriasis?

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Human skin is known to express a complete renin-angiotensin system including angiotensin AT1 and AT2 receptors. Thus, human skin is source of and target organ for angiotensin-II. Angiotensin AT2-receptor (AT2R) stimulation has been described in non-cutaneous cells to promote anti-proliferation and differentiation. Therefore, we hypothesized that AT2R stimulation may be a putative pharmacological concept for the treatment of psoriasis. As a first approach to evaluate this hypothesis, we examined the effect of direct AT2R stimulation on proliferation and differentiation of human primary keratinocytes *in vitro* using the novel non-peptide, orally active AT2 receptor agonist compound 21(C21). Proliferation was determined by 5-bromo-2-deoxyuridine (BrdU) incorporation after stimulation of cells for 24 h with C21 at various concentrations (100 nM, 500 nM, 1 μM). Real-time RT-PCR and Western blot analysis were used to investigate the mRNA and protein expression of early (keratin 10) and late epidermal differentiation markers (involucrin, transglutaminase-1) after a 1- to 4-day treatment with C21 at two concentrations (100 nM, 1 μM). C21 significantly inhibited keratinocyte proliferation in a concentration-dependent manner. Treatment of keratinocytes with C21 significantly down-regulated keratin 10 and up-regulated the expression of involucrin and transglutaminase-1, both on mRNA and protein level. From these results we conclude that angiotensin AT2 stimulation should be further explored as a novel pharmacological concept for the treatment of psoriasis.

P027

Differential regulation of antimicrobial peptide expression in psoriasis by vitamin D analogs

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Antimicrobial peptides (AMPs) are strongly expressed in lesional skin in psoriasis and play an important role as pro-inflammatory 'alarmins' in this chronic skin disease. Vitamin D analogs-like, calcipotriol have anti-psoriatic effects on cutaneous inflammation in psoriasis and might mediate this effect by changing AMP expression. In this study, keratinocytes in lesional psoriatic plaques showed decreased expression of the AMPs- defensin (HBD) 2 and HBD3 after topical treatment with calcipotriol. At the same time, calcipotriol normalized epidermal hyper proliferation and decreased interleukin (IL)-17A, IL-17F and IL-8 transcript abundance in lesional psoriatic skin. In contrast, cathelicidin antimicrobial peptide expression was increased by calcipotriol while psoriasin expression remained unchanged. In cultured human epidermal keratinocytes the effect of different vitamin D analogs on the expression of AMPs was further analyzed. All vitamin D analogs tested blocked IL-17A induced HBD2 expression by increasing IB-á protein and inhibition of NF-B signaling. At the same time vitamin D analogs induced cathelicidin through activation of the vitamin D receptor and MEK/ERK signaling. These studies suggest that vitamin D analogs differentially alter AMP expression in lesional psoriatic skin and cultured keratinocytes. Balancing AMP 'alarmin' expression might be a novel goal in treatment of chronic inflammatory skin diseases.

P028

Characterization of the Notch signaling pathway in melanocytic tumors of the skin

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The NOTCH-signaling pathway has been shown to be of critical importance for the embryonic development and the growth of human melanocytes. We have analyzed the immunohistochemical staining pattern of NOTCH receptors 1, 2 and its corresponding ligand Jagged 1 in invasive malignant melanoma ($n = 25$) and in benign acquired melanocytic nevi ($n = 25$) with special regard to the histopathological subtypes of melanoma and nevi. Additionally, we investigated expression of NOTCH receptor 1 and its corresponding ligand jagged 1 in a vitamin D - sensitive human melanoma cell line (MeWo) using real time PCR and western analysis. We were able to show a distinct expression of NOTCH 1, 2 and Jagged 1 *in vivo* in tissue slides of invasive malignant melanoma and benign common melanocytic nevi as well as on the RNA and protein levels *in vitro* in MeWo cells. In conclusion, our results point at across talk between vitamin D - and NOTCH - signaling pathways while regulating the growth of melanoma cells. Moreover, we conclude that both vitamin D - analogs and pharmacologic modulation of NOTCH - signaling may open new therapeutic perspectives for the treatment of malignant melanoma.

P029

Photo protective effects of 1, 25-dihydroxyvitamin d3 on ultraviolet b- and low-dose ionizing radiation-induced damage in human keratinocytes: *in vitro* analysis of cell viability/proliferation, DNA -damage and -repair.

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We investigated the capacity of 1, 25-dihydroxyvitamin D3 (1, 25(OH) 2D3) to protect human keratinocytes (HaCaT) and squamous cell carcinoma cell lines (SCL-1) against the hazardous effects of ultraviolet B (UVB) and ionizing radiation (IR).Human keratinocytes (HaCaT) and squamous cell carcinoma cell lines (SCL-1) were pretreated with 1, 25(OH)2D3 over 48 h and then irradiated once with UVB- or low-dose IR. We evaluated the results of several assays (Colony-forming-unit-culture assay, WST-1 assay and crystal violet assay), comparing cell viability/proliferation in 1, 25(OH) 2D3-pretreated cells with controls that were pretreated with the carrier substance ethanol alone. Additionally, we analyzed the effects of 1, 25(OH) 2D3 on induction and removal of UV- and IR-induced DNA-damage by detection of cyclobutane pyrimidine dimers (CPDs) via dot blot analysis and äH2AX-foci viaimmunofluorescence in HaCaT-keratinocytes. We proved that 1, 25(OH)2D3, in a concentration of 10–7 M, protects human keratinocytes (HaCaT) as well as squamous cell carcinoma cell lines (SCL-1) against the hazardous effects of UVB-radiation(100 J/cm^2–1000 J/cm^2) *in vitro*. Moreover, it could be demonstrated that the number of CPDs induced in HaCaT-keratinocytes after irradiation with UVB (100 J/cm^2–1000 J/cm^2) was decreased after pretreatment with 1, 25(OH)2D3.Analysis of the time course revealed that the elimination of UVB-induced DNA-damage in HaCaT-keratinocytes occurs quicker when cells are pretreated with1, 25(OH)2D3 (as compared to controls). Concerning low-dose IR up to 2Gy, our findings point to the fact that pretreatment of HaCaT-keratinocytes with 1, 25(OH) 2D3 (10-7M) for 48 h reduces the presence of äH2AX-foci. To put it in a nutshell, our data support the hypothesis that 1, 25(OH) 2D3 protects cultured human keratinocytes against the hazardous effects of UVB and low dose IR.

P030

Alpha-MSH induces expression of suppressors of cytokine signaling (SOCS) 1/3 in human melanocytes

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Suppressors of cytokine signaling (SOCS) are a family of proteins which negatively regulate cytokine signaling. Expression of SOCS1/3 has been found to be induced by a variety of growth factors and cytokines including interleukin (IL)-1, IL-6, tumor necrosis factor-alpha and transforming growth factor-beta (TGF-beta). Of note, the latter cytokines are typically produced by ultraviolet B (UVB) irradiation of epidermal keratinocytes and suppress melanocyte activity (melanogenesis and proliferation).Since UVB also induces alpha-melanocyte-stimulating hormone (alpha-MSH) within the epidermis we wondered whether this peptide may act as a 'master coordinator' orchestrating the melanocyte response in presence of the above cytokines towards a melanocyte-activating direction. As a first step towards this aim, we examined if alpha-MSH induces expression of SOCS1/3 expression in human melanocytes (NHM). Alpha-MSH time- and dose-dependently increased mRNA expression of SOCS1/3 in NHM as well as of SOCS1 in murine B16 melanoma cells. This inductive effect of alpha-MSH on SOCS1 expression in NHM was confirmed by Western immunoblotting. Pre-incubation of the cells with actinomycin D revealed that the inductive effect of alpha-MSH on SOCS1/3 expression is mediated via transcriptional induction. Pathway inhibition studies with the PKA-inhibitor H89, the MEK-inhibitorPD98059, and the adenylate cyclase-blocker SQ22356 further indicated a cAMP-MAPK-MEK-dependent mechanism of the alpha-MSH-mediated induction of SOCS1/3 in NHM. In accordance with these findings, in silico promoter analysis disclosed several putative CREB binding sites within the promoters of both SOCS1and SOCS3 genes. Interestingly, pre-incubation of NHM with alpha-MSH or forskolin, attenuated TGF-beta1-induced phosphorylation of SMAD3 and also abrogated TNF-alpha-induced nuclear translocation of NF-kappaB/p65. In accordance with these findings, pre-incubation of B16 melanoma cells with alpha-MSH or forskolin suppressed the TGF-beta1-mediated inhibition of melanin synthesis. Our findings show for the first time that alpha-MSH induces SOCS1/3 in melanocytes. Via induction of SOCS, alpha-MSH may direct the behavior of melanocytes exposed to a battery of cytokines after UVB exposure into a coordinated, unidirectional but protective response.

P031 (V32)

Tropisetron reduces tissue fibrosis in a mouse model of scleroderma and attenuates collagen synthesis in human dermal fibroblasts possibly via a cAMP-PKA-dependent mechanism

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Many observations point towards a role of serotonin (5-Hydroxytryptamin, 5-HT) or 5-HT-mediated pathways in the pathogenesis of fibrotic diseases. 5-HT is a neurotransmitter, which exerts its biologic effects via at least 15 different receptor-subtypes. Tropisetron (TRO), a 5-HT3-receptor antagonist, has been suggested to improve symptoms of progressive systemic sclerosis in humans. TRO treatment also appears to have beneficial effects in diseases such as rheumatoid arthritis and fibromyalgia. Here, we investigated the effect of TRO on transforming growth factor-beta1 (TGF-beta1) - and bleomycin (BLM)-induced collagen synthesis inhuman dermal fibroblasts (HDF). For *in vivo* studies, we used the BLM mouse model of scleroderma to investigate the effect of TRO on tissue fibrosis. Our data disclose a potent and dose-dependent suppressive effect of TRO on both TGF-beta1- and BLM-induced collagen expressions in HDF. The significance of these findings was corroborated *in vivo* as TRO significantly reduced cutaneous fibrosis induced by BLM. Signal transduction studies exploring the cAMP-PKA and MEK-MAPK pathways as well as SMAD signaling strongly suggested a cAMP-PKA-dependent mechanism of the TRO-mediated suppression of TGF-beta1-induced collagen synthesis in HDF. Interestingly, expression of 5-HT3-receptors was undetectable although 5-HT1B, 5-HT2A/B and 5-HT7 receptors were present in HDF suggesting that TRO presumably acts by a 5-HT3 receptor-independent manner. In summary, our results highlight a potent anti-fibrogenic activity of TRO and shed light into the possible mechanism of TRO-mediated effects in HDF. These data are encouraging for a detailed investigation of the 5-HT system in fibrotic skin diseases and further more may point to novel therapies in the treatment of patients with such disorders.

P032

Detection of novel pro-hormone convertases in normal and transformed human melanocytes. A role in melanogenesis, tumor growth and/or metastasis?

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The Ca2+-dependent pro-hormone convertases (PCs) are a large family of serine proteases which include PC1/PC3, PC2, PC4, paired basic amino acid-cleaving enzyme 4 (PACE4), PC5, PC7, subtilisin kexin isoenzyme-1 (SKI-1) and neural apoptosis regulated convertase-1. Until now, the role of PCs, especially PC1/3 andPC2, in pigment cells has been mainly investigated in the context of processing of pro-opiomelanocortin, the precursor for melanocyte-stimulating hormone (MSH).However, there is increasing evidence that PCs may play an important role in cellular transformation, acquisition of a tumorigenic phenotype and metastasis. Accordingly, we recently identified PACE4 as a novel PC over expressed in human melanomas. Here, stable transfection with rPACE4 lead to increased expression of matrix metalloproteases and enhanced cellular invasion (Böhm et al., in preparation). To check if additional members of the PC family are altered in melanoma we performed expression analysis of SKI-1, PC5/6 and PC7 employing normal human melanocytes (NHM) and a panel of 9 human melanoma cells lines derived from different stage of diseases progression. Expression of these PCs was examined by conventional RT-PCR, real-time RT-PCR and western immuno blotting as well as by immunofluorescence analysis. Constitutive expression of SKI-1 was detected in NHM as well as in all tested melanoma cell lines without any significant difference in the protein levels of this PC between normal and transformed cells. Interestingly, PC5/6 expression at the mRNA level was up to 124, 93 fold higher in melanoma cells than in NHM. Conversely, PC7 mRNA levels were significantly lower in the tested melanoma cell lines compared with NHM. Importantly, in 6 out of 9 melanoma cell lines, PC5/6protein expression was higher than in NHM. Immunofluorescence analysis disclosed a cytoplasmic localization of PC5/6 in melanoma cells. Interestingly, alpha-MSH up-regulated mRNA expression of all tested PCs in a time-dependent manner in NHM. These preliminary data indicate that in addition to PACE4 other members of the PC family are altered in their expression in transformed melanocytes *in vitro*. Functional studies employing gene knock-down of PC5/6 or over expression of PC7 are currently underway to investigate the relevance of these findings. Moreover, *in situ* analysis of these PCs in melanoma tissue samples will be useful to test if expression of any of these PCs could serve as a prognostic marker.

P033

A new horizon in hair keratin regulation: the importance of neuroendocrine hormones as transcriptional mediators of hair keratins and keratin-associated proteins

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Hair keratins and keratin-associated proteins (KAPs) constitute the major building blocks of the hair. Although endocrine regulation for several keratins has been shown before, up to now, our knowledge on the most complex regulation of hair keratin gene expression is limited. We investigated the possible regulation of the neuroendocrine hormones prolactin (PRL), thyroid stimulating hormone (TSH) and thyrotropin regulating hormone (TRH) on hair keratin and KAP gene expression. Human female scalp hair follicles (HFs) were cultured for 24 h with TSH, TRH or vehicle and for 48 h with PRL or vehicle. Microarray analysis was used in order to detect differentially regulated hair keratin and KAPs genes. Quantitative real time PCR was performed on KRT35, KRT31 and KRT32 genes, expressed in the hair forming compartment and representing its major areas, i.e. matrix/pre-cortex, cortex and cuticle, respectively. In addition, qPCR of MSX2, a major regulator of hair keratin expression, was also performed for TSH and TRH treatments. Since keratins K15and K19 are prominently expressed by the HF epithelial stem cells, we also tested the regulation of these epithelial keratins by PRL. Microarray analysis revealed a large set of hair keratin and KAP genes to be regulated by PRL, TSH and TRH. By qPCR, TSH down regulated KRT31 and KRT32 genes, while TRH down regulated KRT32. Congruently, MSX2 expression was down-regulated by both TSH and TRH. PRL up-regulated expression of KRT15 and KRT19 but down-regulated KRT31expression. In summary, hair keratin and KAPs genes are regulated by PRL, TSH and TRH. This may eventually lead to changes in hair properties. In view of their essential role in hair formation and phenotype, hair keratins and KAP regulation by endocrine controls is of critical importance and should develop into a new frontier in hair research.

P034

Thyrotropin releasing hormone and 17-ß-estradiol regulate intra-cutaneous prolactin and prolactin receptor expression in human skin and hair follicles; preliminary evidence using serum free organ culture.

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Although it is clear that human scalp hair follicles (HFs) are both a non-classical target and an extra-pituitary source of prolactin (PRL) production, a potent hair growth modulator, the regulation of PRL and PRL receptor (PRLR) expression in human skin and HFs is unknown. Using the serum free organ culture model we investigated whether the potent stimulators of pituitary PRL secretion, thyrotrophin releasing hormone (TRH) and 17-ß-estradiol (E2), also modulate intra-cutaneous PRL and PRL receptor (PRLR) expression. Full-thickness front temporal female scalp skin or micro dissected anagen VI HFs, were exposed in serum-free organ culture for 6 days to 100 nM E2, TRH (1, 5, 10 ng/ml) or vehicle control only. Cryosections of TRH-treated HFs, and of E2-treatedfull-thickness human scalp skin were assessed for PRL and PRLR immunoreactivity by quantitative immunohistomorphometry of selected skin and HF compartments. qPCR for PRL and PRLR was also performed on E2-treated, cultured human outer root sheath (ORS) keratinocytes. In TRH-treated HFs, PRL immunoreactivity was significantly up-regulated in the ORS ($P < 0.05$), corresponding to an increase in PRL transcription in hair follicles measured by qPCR. ORS PRLR immunoreactivity was significantly down-regulated by TRH ($P < 0.05$). In E2-treated skin, PRL immunoreactivity was significantly up-regulated ($P < 0.05$) in the epidermis and the HF ORS. PRLR immunoreactivity *in situ* was significantly up-regulated in sebaceous and eccrine sweat glands ($P < 0.05$). qPCR showed significant up-regulation of both PRL and PRLR transcription in ORS keratinocytes ($P < 0.001$). Although extra-pituitary PRL production is driven by an alternative up-stream promoter, our preliminary evidence suggests that intra-cutaneous regulation of PRL expression may respond to the classical pituitary controls of PRL secretion. Targeting the key regulators of intra-cutaneous PRL and PRLR expression may be a treatment option to target prolactin mediated skin disease and hair disorders.

P035

Frog skin organ culture - a model system for wound healing

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Amphibians possess some remarkable peptides in their skin which may be beneficial to wound healing, not only in the amphibian system, but also in mammalian species. Therefore frog skin may have to offer clinically relevant lessons for how to promote wound healing in human skin. Anuran amphibian tadpoles can regenerate lost limbs, while adult amphibians lose this capability post metamorphosis. However, studies on wound healing and re-epithelisation in adult frog skin are largely lacking. Therefore, we have developed a modified frog skin organ culture system that allows assessment and manipulation the amphibian skin wound healing response. Dorsal skin from adult male and female *Xenopus tropicalis* frogs was harvested from sacrificed animals and 3 mm punch biopsy holes were made in the skin, around which a further 6mm piece of skin was punched out. These skin fragments were placed dorsal side up in 24 well culture plates are cultured up to 7 days at 25°C in either Williams E medium (50% dilution with dH_2O) alone or with the addition of the following exogenous wound healing promoting factors; frog serum, TRH (thyrotropin releasing hormone) and bombesin. Re-epithelisation was assessed morphologically and histochemically. Results demonstrate that re-epithelisation of the wound began already at day 1 and was increased dramatically with the addition of 5% frog serum by day 3 and day 7 (% area reduction: d3 27.5% + 7.5, d7 70% + 8.5 cf. controls d3 7.25% + 1.20, d7 8.23% + 3.8). The re-epithelisation sheet typically had the appearance of a simple squamous epithelial sheet showing a distinct 'paving stone' pattern. Bombesin and TRH, two neuropeptides found in high concentrations in frog skin, also stimulated re-epithelialization and wound closure compared to vehicle control tissue. This study reports a simple, pragmatic, and instructive organ culture system for the study of frog skin wound healing under highly defined experimental conditions, and demonstrates its usefulness as a discovery tool for identifying novel candidate promoters of wound repair in dermatological sciences. Financial support for this project was provided by the British research council (BRC).

P036

A novel neuroendocrine control of human mitochondrial activity and biogenesis *in situ*: TRH and TSH power mitochondria in human epidermis

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Here, we demonstrate that thyrotropin (TSH) as well as thyrotropin-releasing hormone (TRH) up-regulate mitochondrial activity, efficacy and capacity in adult human epidermis *in situ*. Normal, organ-cultured human skin provides the first evidence that treatment with TSH and TRH strongly up-regulates the number of light-microscopically visualized, MTCO1-immunoreactive intracellular 'dots' in the epidermis labeled by a specific, mitochondria-selective antibody raised againstMTCO1, which selectively demarcates mitochondria. Transmission electron microscopy confirms on the ultra structural level that TSH and TRH indeed stimulate mitochondrial proliferation and biogenesis. Additionally, we are exploring whether these enzymological, transcriptional and ultra structural indications of increased mitochondrial energy metabolism actually translate into increased oxygen consumption by TSH-treated human skin. A first, as yet unconfirmed pilot experiment suggests that this is indeed the case. These studies pioneer the concept of a surprisingly potent, unexpected neuroendocrine control of mitochondrial energy metabolism and biogenesis based on transcriptional, ultra structural, immunohistochemical and biochemical evidence. In addition, we introduce human skin organ culture as a physiologically relevant research tool for further exploring this important novel control of mitochondrial biology.

P037

Endocrine modulation of human hair follicle epithelial progenitor cells *in situ* and *in vitro*: Effects of thyroid hormones, TSH, and calcitriol

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Cloning of the human K15-promotor into a non-retroviral GFP/geneticin-resistance expression system and Lipofectamine®-mediated transfection of micro dissected, organ-cultured adult human scalp hair follicles generates specificK15-promoter-driven GFP expression in their stem cell-rich bulge region. Vital progenitor cells can subsequently selected by their green fluorescence, by adhesion to collagen type IV and fibro nectin, and by their genetic in resistance. Using this novel system we provide the first evidence that thyroid hormones (T3, T4) strongly up-regulate K15 promoter activity, K15 transcription and protein production in normal adult human hair follicle epithelial progenitor cells *in situ* and *in vitro*, and show that these cells underlie profound hormonal regulation. Moreover, thyroid hormones reduce strongly the colony forming efficiency (CFE), proliferation, cell number and viability and induce apoptosis in cultured human K15-GFP+ cells. Under the influence of thyroid hormones, K15-GFP+ cells also start to express the TSH-beta-receptor and thus become sensitive to stimulation with TSH. After stimulation of these cells with TSH, they started to differentiate and express e.g. keratin 6. For comparison, we also analyzed the effects of calcitriol. This steroid hormone also induced morphological changes, up-regulated VDR and CD200 expression, and impaired (CFE), in K15-GFP+ cells. These data provide novel insights into the as yet largely obscure (neuro-)endocrine controls of normal human epithelial progenitor/stem cells in general, and illustrate how primary human K15-GFP+ cells can be exploited to further elucidate these crucial controls.

P038

Impact of VDR micro RNA expression and epigenetic silencing on anti-proliferative effects of 1,25-dihydroxyvitamin D3 in malignant melanoma cells.

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Malignant melanoma cells express the vitamin D receptor (VDR) and respond to the anti-proliferative activity of 1, 25-dihydroxyvitamin D3 (1,25(OH)2D3). However, some melanoma cell lines fail to respond to the anti-proliferative effects of vitamin D. We have previously studied effects of 1,25(OH)2D3 on the growth of seven melanoma cell lines. While three cell lines (MeWo, SK-Mel28, SM) responded to anti-proliferative effects of 1,25(OH)2D3, the others (SK-Mel5, SK-Mel25, IGR, MelJuso) were resistant. In the present study, we analyzed whether vitamin D signaling in vitamin-D-resistant melanoma cells is abrogated by mutations in the VDR gene, VDRmicroRNA (miRNA) expression, or by epigenetic silencing. To exclude mutations in the VDR gene the VDR cDNA was sequenced in vitamin D-resistant melanoma cells. Recent studies have demonstrated that VDR expression is regulated post-transcriptionally by miRNA. A potential miRNA recognition element (MRE125b) was identified in the 3'-untranslated region of human VDR mRNA. We now investigated whether MRE125b is differentially expressed in vitamin D-sensitive as compared to -resistant melanoma cells. In different types of cancer, numerous genes are regulated via epigenetic events. In malignant melanoma, at least 50 genes have been identified to date to be epigenetically silenced during disease development and progression by promoter hyper-methylation. In this study we investigated whether epigenetics effects are of importance for the abrogation of vitamin D signaling in vitamin D-resistant melanoma cells. We used the de-acetylase inhibitors (HDACI) trichostatin A (TSA) to elucidate the effect of protein acetylation on cell cycle progression and survival. Additionally, we analyzed effects of 5-azacytidine. By using5-azacytidine, a DNA methyl transferase inhibitor (DNMTI), the hyper-methyation ofDNA can be avoided, so that transcriptionally activation of genes can be ensured. In conclusion, our data indicate that both VDR miRNA expression and epigenetic silencing are of importance for the abrogation of vitamin D signaling in vitamin-D-resistant melanoma cells.

P039

Cross-talk between Vitamin D- and NOTCH- signaling pathways in cultured human keratinocytes and sebocytes

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NOTCH- and vitamin D- signaling pathways, that both depend on the presence or absence of several specific receptor proteins and corresponding ligands, are of high importance for the embryonic development and the growth of human skin cells, including keratinocytes and sebocytes. We investigated the expression of key components of the NOTCH-signaling pathway (NOTCH receptors 1, 2, 3, 4; corresponding ligands delta, jagged) in cultured human keratinocytes (HaCaT, SCL-1) and sebocytes (SZ95) using real time PCR and western analysis. We found strong expression of NOTCH 1 and jagged 1 on the RNA and the protein levels in all cell lines analyzed. *In vitro* treatment of SZ95 sebocytes with 1, 25-dihydroxyvitaminD3, the biologically active form of vitamin D, resulted at low concentration (10–10 M) in elevated RNA expression of jagged 1 and NOTCH 1. Interestingly, treatment with1, 25-dihydroxyvitamin D3 modulated expression of key components of the NOTCH signaling pathway differentially in spontaneously immortalized and non-malignant HaCaT keratinocytes as compared to the cutaneous squamous cell carcinoma cell line SCL-1. Treatment of HaCaT cells with 1, 25-dihydroxyvitamin D3 in high concentration (10-6 M) resulted *in vitro* both in inhibition of cell proliferation and inreduced RNA and protein expression of jagged 1. RNA expression of Notch 1 was inhibited as well, while protein content was only marginally affected. In SCL-1 cells, RNA expression of NOTCH 1 was slightly reduced after treatment with1, 25-dihydroxyvitamin D3 in high concentration (10-6 M), while protein content was only marginally altered. In conclusion, our results point at a cross talk between vitamin D- and NOTCH- signaling pathways while regulating the growth of keratinocytes and sebocytes. Our findings point at a differential response of non-malignant and malignant keratinocytes to the biologic effects of vitamin D analogs that involve the NOTCH - signaling pathway. Moreover, we conclude that both vitamin D-analogs and pharmacologic modulation of NOTCH-signaling may open new therapeutic perspectives for the treatment of hyper-proliferative skin diseases and sebaceous gland disorders.

P040

Dissecting the role of substance P in splenic immune regulation reveals control of peripheral inflammatory processes by enhanced neuroimmune communication

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Allergic dermatitis (AD) is characterized by an imbalance of TH1/TH2-immuneresponses. Acutely experienced stress strongly influences this imbalance due to a change in local neuro-immune communication. The sensory neuropeptide Substance P (SP) acts as an important stress mediator in this scenario, with its own stress axis in the skin, modulating mast cell as well as dendritic cell activation. Here we postulate that SP-dependent neuro-immune modulation occurs also in the spleen and potently alters the stress and AD relevant cytokine and immunocyte-milieu. To address this hypothesis, AD was induced in C57BL/6 mice by sensitization to and intra-dermal challenge with ovalbumin. Animals were additionally exposed to noise stress for24 h prior to challenge. In this model stress leads to hyper-innervation and increased numbers of antigen-presenting cells (APC) in splenic white pulp. Moreover, we observe an altered NK-1- and PPT-1- mRNA expression and at the same time an increase in APC and T-regulatory cells expressing the NK-1 receptor. These findings were accompanied by an altered TH2/TH1 (IL-4, IL-5 = TH2; IFN-γ, TNF-alpha= TH1) cytokine production on mRNA level. AD alone had no effect on splenic IL-10and IL-12 mRNA expression while stress alone lead to an increase of proinflamatoryIL-12 and stress in AD lead to an increase of regulatory IL-10 mRNA compared to the control. Interestingly only in stress groups the level of IL-2 mRNA increased compared to control. Moreover, all observed changes were potently reversed by pharmacologically antagonizing the SP receptor NK1 *in vivo*. *In vitro* SP had the capacity to raise the quantity of MHC-II expression on splenic CD11c+/CD8+ DCAPC#s and alter their cytokine production towards a TH2 profile, while on CD3+/CD4+ T-cells SP raised the percentage of CD25 expressing cells and promoted a dose dependent TH1shift. Comparing these findings and keeping in mind that besides stress, AD is a biochemical stressor itself for the splenic immunologic micro-environment; we conclude that the AD 'reaches' the spleen and SP is a key player of splenic stress induced regulations. Further investigations have to reveal the impact of this splenic stress-response in determining allergic inflammation, facilitating tolerance and potentially suppress cutaneous inflammation.

P041

Acute and chronic stresses differentially affect exacerbation of allergic dermatitis

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Stress has long been expected to induce and/or exacerbate allergic diseases such as atopic dermatitis (AD). However, published data to date is full of inconsistencies especially with respect to acute versus chronic conditions. The acute state of AD is characterized by a prominent expression of so called TH2- cytokines while chronification of AD is marked by an additional increase of TH1 cytokines. Interestingly, TH1 cytokines are also increased after acute stress exposure while TH2cytokine predominance is characteristic for chronic stress experience. To investigate and compare the effect of acute versus chronic stress in AD we employed a combined mouse model of experimental AD and stress. AD was induced in C57BL/6mice by double sensitization and intra-dermal challenges using chicken egg ovalbumin.Animals were additionally exposed to sound as well as restraint stress for two h prior to challenge. To induce an acute stress response sound and restraint stress were applied only once, to induce a chronic stress response sound and restraint stress were applied daily over 5 days. To understand the role of the key cutaneous neuropeptide stress mediator substance P (SP) in this experimental setting another group of mice were treated with SP instead of stress. Interestingly, we found that chronic stress reduces epidermal thickness in control as well as AD skin while acutely as well as chronically administered SP increased epidermal thickness. Chronic stress also increased the number of mast cells both in control and AD skin while SP did not. With respect to eosinophil infiltration chronic stress reduced eosinophil numbers in the dermis. As measured by semi-quantitative PCR our results show that stress shifted the cytokine profile towards the expression of TH1 cytokines while stress combined with AD did not. Together our data suggest that chronic stress exposure promotes an anti-allergic cytokine milieu associated with reduced inflammation and epidermal thickening, however, this pathway does not involve SP. Further investigations will clarify possible mechanisms.

P042

Profiling the aging process: Genetics of the graying human hair follicle

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Quality of life in our aging society depends crucially on healthy aging. One obvious hallmark of aging is hair graying. The graying hair follicle here offers itself as an easily accessible model tissue to dissect the aging process and determine worthy targets for regenerative intervention. We here present intriguing new data obtained from intra-individual sample comparison. Mapping the graying process we calculated differential gene expression between pigmented and gray and pigmented and white human scalp skin hair follicles from identical donors. On average 2680.0 probe sets were down-regulated in gray follicles and 2390.6 in white. 2513.0 and 2578.4 probe sets were up-regulated in gray and white follicles respectively. Intersections for mend from five donors of one pigmentation status revealed 194 probe sets were down-regulated comparing pigmented versus gray and 192 comparing pigmented versus white. Up-regulated were 186 pigmented versus gray and 305 pigmented versus white. A list of 273 candidate genes that protect, regulate and terminate melanocyte #s life cycle and melano-genesis discovered 263 genes represented on the used array platform by 674 probe sets. Among the higher and lower expressed genes we found representatives of these genes: up-regulated were three genes represented in gray follicles and five in white. Among the 194 probe sets down-regulated gray follicles were 15 of our pigmentation list. Among the 192down-regulated ones in white hair follicles we already found 22. Higher or lower expression provided inside in possible biological pathways that are involved in the graying process. We found for example regulation of melanogenesis-associated genes (tyrosinase-related protein 1, TYRP1; tyrosinase, TYR; silver homolog; SILV)that implicated a down-regulation of the melano-genetic pathway. In addition, regulation of v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT) or endothelin receptor type B (EDNRB) hint at hampered cell migration and reduction of hair follicle ability to give rise to new melanocyte progeny. Together, these findings prove the aging hair follicle to be a valid model for tissue specific aging facilitating intra-individual comparison as there are pigmented, gray and white hair follicles found in most aging scalp skin samples.

P043

Mitochondrial metabolism and DNA integrity - novel targets for alpha-MSH on human keratinocytes and melanocytes after UVB irradiation

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It is widely accepted that melanin synthesis induced by alpha-MSH protects from nuclear genotoxic stress and from reactive oxygen species (ROS) upon UVB irradiation. However, the effects on UVB and alpha-MSH on mitochondrial metabolism and mitochondrial (mt) DNA integrity remain largely unexplored. Here, we show that in both human keratinocytes and melanocytes, UVB irradiation reduced the level of nuclear respiratory factor-1 (NRF-1), a nuclear-encoded key transcription factor orchestrating mitochondrial gene expression of such as cytochrome C. Alpha-MSH alone induced NRF-1 expression and attenuated UVB-induced reduction of the relative amounts of NRF-1 suggesting a protective effect of the hormone on mtDNA integrity. However, while physiological doses of UVB (5-15 mJ/cm2) had no detectable effect on the overall mtDNA copy number and the presence of the common deletion, a marker of mtDNA damage induced by oxidative stress, alpha-MSH surprisingly increased the number of deleted copies of mtDNA compared with UVB alone. The increase in the number of deleted copies of mtDNA by alpha-MSH was only detected in melanocytes but not in keratinocytes where the hormone had some protective but not photo-protective effects. Our data indicate that α-MSH albeit broadly regarded as a cyto-protective agent actually increases mtDNA damage. This effect of alpha-MSH on mtDNA integrity is independent ofNRF-1 induction and presumably mediated by increased melanin synthesis and production of reactive oxygen species during the melanogenesis specifically in melanocytes.

P044

Stress increases IL-10+ nerve fiber numbers and reduces the INFγ+ cells in an allergic inflammation mouse model

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Mast cells (MC) and nerve fibers (NF) are important factors in the effect of psycho emotional stress on allergic diseases such as atopic dermatitis by releasing pro-inflammatory mediators such as MC proteases, cytokines or neuro-peptides during an acute stress response. However, recent studies show that under certain conditions MC exert anti-inflammatory capacities producing and releasing e.g. IL-10and proteases that terminate stress neuropeptide signaling. Moreover, new findings show that the effect of MC are strictly time dependent and that they act pro- or anti-inflammatory depending of the time point after induction of inflammation or on the stress-paradigm employed. We therefore hypothesize that in contrast to the pro-inflammatory role MC play during the initial steps of stress-exacerbated inflammation, they may contribute to an early termination of an inflammatory response especially under chronic stress conditions. To answer the question if specific stress settings can stimulate MC to act anti-inflammatory, we employed a combined mouse model of experimental allergic dermatitis (AD) and repeated stress-exposure prior to sensitization. AD was induced in C57BL/6 mice by double sensitization (s.c.) and an intra-dermal challenge using chicken egg ovalbumin. Animals were additionally exposed to sonic stress (24 h) prior to each sensitization episode. 48 h after the provocation the affected back-skin of the mice was harvested and prepared for cryo-sectioning. Earlier we were able to demonstrate reduced inflammation in the skin of stressed AD mice in this setting. Quantitative histomorphometry was now used to assess the cytokine profile at the site of inflammation. Surprisingly we found expression of the anti-inflammatory cytokineIL-10 in NF and this expression was down-regulated in AD skin (in a ratio 3:1$P < 0.01$). Stress in addition to AD normalized IL-10+NF numbers. By contrast, the number of INF γ+ cells was increased in the AD group ($P < 0.01$) while stressed AD animals show a reduction of INF γ+ cells (in a ratio of 3:1; $P < 0.01$).Interestingly, cell contacts between MC and INF γ+ cells (In a ratio 3:1 $P < 0.01$)was augmented in this group in comparison with the AD group. In summary we here show surprisingly altered expression of IL10 and IFN γ in NF during chronic stress induced reduction of allergic inflammation indicating an anti-inflammatory role for cytokine releasing NF and possible direct regulation of IFN γ expression by anti-inflammatory MC. Further investigations are required to determine the exact phenotype and role of anti-inflammatory MC in the cutaneous stress response.

P045

Does prolactin exert gender- and/or site-specific effects in human skin?

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The polypeptide neurohormone prolactin (PRL) has multiple different effects on skin function, which vary between species. In humans, we have demonstrated that PRL exerts a catagen promoting effect on human male occipital hair follicles (HFs).However, given the sexual dimorphism in PRL levels, we hypothesized that PRL may exert gender- and/or site-dependent effects in human skin and that HFs may offer a sensitive and instructive test system for exploring this hypothesis. Frontotemporal female scalp skin anagen VI HFs were obtained from 2 healthy females undergoing routine facelift procedures. These were cultured in serum free organ culture medium in the presence of 400ng/ml PRL and a pure PRL receptor (PRLR) antagonist. HF growth and hair cycle stage were measured by histomorphometry, and gene expression profile was assessed by DNA micro array analyses. PRL treatment of female frontotemporal scalp HFs significantly increased hair shaft elongation compared to vehicle controls ($P < 0.01$). This was partially reduced by the addition of PRLR antagonist. There was no increased TUNEL positivity; indicating that PRL did not induce catagen in these HFs. This was independently confirmed by analysis of hair cycle scores. Microarray data demonstrated down-regulation of catagen associated genes, including BMP2 and 4, and several site and/or gender specific changes in gene regulation. These observations provide preliminary evidence in favor of the novel hypothesis that PRL exerts as yet under-appreciated gender- and/or site-specific effects on human HF growth. This novel hypothesis may explain why hyper-prolactinaemia is associated with both hirsutism and hair loss in females, and with reduced facial and body hair growth in males. Our pilot data highlights the importance of a systematic re-examination of gender-and/or site-specific effects of PRL in human skin.

P046

Selective up-regulation of antimicrobial proteins in skin samples after insect bites

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Insect bites from mosquitoes or other bugs are a common problem leading to toxic or allergic reactions, inflammation, itching and pain. Infections occur only occasionally, although stinging disrupts the permeability barrier and may allow the invasion of bacteria that colonize the insect's spine or skin surface. We ask whether antimicrobial proteins are responsible for the relatively low rate of infection after insect bites. The expression of various antimicrobial proteins (human beta defensin (hBD)-2 and -3, human neutrophil peptide (HNP)-1-3, RNase 7, psoriasin (S100A7) and the cathelicidin LL-37) was determined by immuno-histochemistry in skin samples obtained from insect bite lesions and compared to healthy skin. In addition, H&E and mast cell tryptase stainings were performed. H&E staining revealed a pronounced infiltrate of inflammatory cells, mainly lymphocytes and neutrophils and occasionally eosinophils, in the dermis. In the insect bite channel we predominantly found an infiltrate with neutrophils. The number of mast cells and mast cell tryptase was only moderately increased. The antimicrobial protein expression was variable. Moderately increased staining for hBD-2 and -3, and RNase 7, as well as a pronounced increase of psoriasin was noted in the epidermis adjacent to the insect bite channel. Within the insect bite channel itself and in the adjacent dermis, but not within the epidermis, we found intense staining for LL-37 and HNP 1-3, both derived from neutrophils. In bullous insect bite reactions, staining for LL-37 and HNP 1-3 was found within the blister fluid. Staining for HNP 1-3 and psoriasin, but not LL-37, was also found in the epithelia of the blister floor. In summary, we found a pronounced up-regulation of psoriasin, LL-37 and HNP 1-3 in insect bites. Psoriasin protein expression occurred in the epidermis, whereas HNP 1-3 was identified within the neutrophils in the epidermis and dermis and LL-37 in the dermis. Induction of antimicrobial peptides may therefore help protect against infections after skin barrier disruption and the potential invasion of bacteria after insect bites.

P047

Gene expression analysis reveals a strong increase in FcγR4 expression in the skin of mice after induction of epidermolysis acquisita bullosa

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Objective: Epidermolysis bullosa acquisita (EBA) is an organ-specific autoimmune disease that is associated with tissue injury and fluid accumulation within the skin. The initial trigger for the blister formation is IgG auto-antibodies targeting type VII (anchoring fibril) collagen which is involved in cell-matrix adhesion in the skin. One of the most important factors that determine the activity of autoantibodies is the interaction with their cellular Fc-receptors (FcγR) on innate immune effector cells. Until today no quantitative data are available on the expression of FcγRs in bone marrow, lymph node and skin of EBA diseased mice. In this study we analyzed the mRNA expression of the activating FcγR 1, 3 and 4 and the inhibitory FcγR2b by real-time RT-PCR. The level of expression was quantitatively compared to the increase of innate effector cells in skin lesions.

Methods: EBA was induced in SJL-1 mice by immunizing the mice with recombinant murine type VII collagen (GST-mCOL7C) (R. Ludwig, Department of Dermatology) emulsified in the non-ionic polymeric adjuvant titermax (ALEXIS Biochemicals). 17weeks after immunization the mice were killed and femurs, inguineal lymph nodes and foot pad skins were snap frozen. mRNA expression in bone marrow, lymph nodes and skin was analyzed using real-time RT-PCR.

Results: Our results demonstrate that the expression of FcγR1, 2b, 3 and 4 increased strongly in the skins of EBA diseased mice. FcγR4 transcripts increased about 20.000 fold, FcγR1 mRNA about 200 fold, and FcγR3 about 70 fold. In addition, the inhibitoryFcγR2b increased 50 fold in the skin. Interestingly, the increase in innate effector cells was below a factor of 100. The increase in expression of FcγRs 1, 2b, 3 and 4 inactivated lymph nodes was about 10–50 times lower compared to the expression levels in the skins. No change was detectable in bone marrow.

Conclusion: The highest increase in mRNA transcription was found for FcγR4 after induction of EBA in SJL mice. We conclude that especially the expression of FcγR4 contributes to pathogenesis of murine EBA. Thus, the treatment with drugs for FcγR4 blockade might be considered as potential therapeutics in an established disease.

P048

Apoptosis, differentiation and proliferation: Impact on clinical outcome of patients with interface dermatitis - an immunohistochemical study

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Aims: To analyze the expression of CD95 in comparison to markers of proliferation and differentiation and epidermal cytokeratins on tissue samples from diseases characterized by a cutaneous interface dermatitis.

Methods: We examined 20 tissue samples showing cutaneous interface dermatitis including cutaneous lupus erythematosus; chilblain lupus, systemic lupus erythematosus and discoid lupus erythematosus, lichen ruber, erythema multiforme, dermatomyositis and graft-versus-host disease. Unaffected skin of healthy individuals served as control. All slides were analyzed by conventional microscopy and by immunohistochemistry using antibodies against CD95, Mib1, PCNA, pan-cytokeratin markers and low/high molecular weight cytokeratins. Immunohistochemistry was performed on slides of paraffin embedded tissue.

Results: We could show that all diseases investigated are characterized by a distinct pattern of expression for markers of proliferation and differentiation. Clinical outcome of patients was associated with different expression patterns of the markers investigated.

Conclusions: The ligand of death factor CD95 is important for immunoregulatory functions in human cells, including tumor cell survival. It serves as a co-stimulator during T-cell-activation and is thought to be associated with several inflammatory diseases. We could show a distinct expression pattern of CD95 and other markers of proliferation and differentiation in diseases with affection of the dermal-epidermal zone. Intriguingly, these findings are associated with therapeutic efficacy and may open new insights into pathogenesis and new aspects of therapeutic targets.

P049

Follicular swiss cheese pattern - clue to diagnose of alopecia areata

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Background: Yellow dots are the most useful dermoscopic criterion in the clinical diagnosis of alopecia areata and correspond histopathologically with dilated follicular infundibula. They are found clinically in the vast majority of AA cases and help to differentiate alopecia areata from trichotillomania, telogen effluvium and from scarring alopecias, such as frontal fibrosing alopecia. Histopathology of alopecia areata differs with disease activity. The peribulbar lymphocytic infiltrate as the most pathognomonic change is only found in 40% of cases. The dermatopathologist, therefore, heavily depends on other diagnostic features. We therefore evaluated whether dilated follicular infundibula are a helpful diagnostic sign in the histopathologic evaluation of alopecia areata.

Objective: To determine the frequency of yellow dots, peribulbar lymphocytic infiltrates inflammatory infiltrates of lymphocytes and eosinophils within fibrous streamers and a shift to catagen/telogen follicles in alopecia areata. Histopathologic features of 56 specimens of 33 patients were correlated with the clinical findings and the alopecia areata subtype.

Results: More than 50% of all biopsies showed yellow dots, regardless of horizontal or vertical sectioning of the slides. Yellow dots showed a maximum occurrence in there covery stage of alopecia areata and were seen quite often in AA incognita. The characteristic peribulbar lymphocytic infiltrate was found in a minority of cases

Conclusions: Dilated follicular infundibula are an exceedingly useful criterion in the histopathologic diagnosis of alopecia areata. They are seen in all subtypes of AA, with special emphasis on AA in recovery stage, which shows many overlapping features with androgenetic alopecia. The follicular Swiss cheese pattern is of great help in the daily routine to recognize alopecia areata.

P050

Koebnerisin (S100A15): a novel inflammatory marker in koebnerized psoriasis

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Psoriasis is a highly prevalent chronic inflammatory skin disease of genetic background. First cloned from koebnerized psoriatic skin, S100A15 (koebnerisin) is highly homologous to S100A7 (psoriasin). Both proteins are encoded within the psoriasis susceptibility locus PSORS4 (chromosome 1q21) and are genetically linked to inflammation. S100A15 and S100A7 are almost identical in sequence and difficult to distinguish. We developed specific antibodies and demonstrated their regulation by different cell types in normal skin. Both proteins are expressed by in the differentiated epidermal layers, S10015 but not S100A7 is expressed by melanocytes and Langerhans cells. Both proteins are elevated in chronic inflammatory psoriasis further pronouncing the supra-basal epidermal layers. Compared with S100A7, S100A15 expression highlights 'activated' basal keratinocytes at the epidermal-stromal junction as well as the markedly increased vasculature of the corresponding papillary dermis. Thus, their distinct distribution pattern of S100A7 andS100A15 in inflamed psoriasis reflects their distinct functions as chemo-attractants, which synergize to potentiate inflammation when co-expressed. Regulation, distribution and function of S100A15 distinct from S100A7 require discriminating among these highly homologous proteins in inflammatory skin diseases, skin cancers and beyond.

P051

Targeting NF-κB with a natural triterpenoid alleviates skin inflammation in a mouse model of psoriasis

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Psoriasis vulgaris is a common chronic inflammatory skin disease involving cytokines and an activated cellular immune system. At variance to skin from patients with atopic dermatitis or from healthy subjects, human psoriatic skin lesions exhibit strong activation of transcription factor NF-κB that is mainly confined to dermal macrophages, whereas only a few dendritic cells but no CD3+ lymphocytes show activated NF-κB. Additionally, the biopsy specimens from patients with atopic dermatitis revealed no dermal macrophages exhibiting activated NF-κB. Since NF-κB signaling is required for the induction and/or function of many cytokines and aberrant cytokine expression has been proposed as an underlying cause of psoriasis, we investigated whether NF-κB targeting would affect the course of the disease in theCD18 hypomorphic (CD18hypo) mouse model of psoriasis. When mice with severe psoriasis form lesions were treated systemically or locally with the IκB kinase inhibitor acetyl-11-keto-β-boswellic acid (AKβBA), NF-κB signaling and the subsequent NF-κB-dependent cytokine production as shown by the TNF-α production of macrophages was profoundly suppressed. In addition, application of the compound counter acted the intradermal MCP-1, IL-12 and IL-23 expression in previously lesional skin areas, led to resolution of the abundant immune cell infiltrates, and significantly reduced the increased proliferation of the keratinocytes. Overall, the AKβBA treatment was accompanied by a profound improvement of the psoriasis disease activity score in the CD18hypo mice with reconstitution of a nearly normal phenotype within the chosen observation period. Our data demonstrate that NF-κB signaling is pivotal for the pathogenesis in the CD18hypo mouse model of psoriasis. Therefore, targeting NF-κB might provide an effective strategy for the treatment of psoriasis.

P052

Interleukin (IL)-22, a T-cell mediator, induces in keratinocytes IL-20, a tissue cell mediator: a novel cascade with major relevance for the pathogenesis of psoriasis

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Psoriasis is a common chronic skin disease. Recent studies demonstrated that IL-20 and IL-22, two cytokines of the IL-10 interferon family and produced by keratinocytes and T cells, respectively, inhibit the keratinocyte terminal differentiation and induce psoriasis-like epidermis alterations. Here, we investigated the relationship between these mediators. The analysis of skin lesions from psoriasis patients revealed a highly significant, positive correlation between IL-20 and IL-22 mRNA levels and provoked the questions whether one cytokine induces the production of the other. Whereas IL-20 was not able to regulate IL-22 production, IL-22 indeed induced IL-20 mRNA and protein in human keratinocytes from conventional cultures and reconstituted epidermis. However, IL-22 had only a minimal effect, if any, on other IL-10 - interferon family members including IL-19 and IL-26. Cutaneous IL-20was also elevated in mice following IL-22 application. Accordingly, some of the IL-22effects on differentiation regulating genes that are essential for psoriasis-like epidermis alterations were partially mediated by an endogenous, secreted protein and attenuated by anti-IL-20 antibodies. Like IL-22, IL-17A and TNF-α induced IL-20 in keratinocytes, whereas IFN-γ and IL-20 itself did not. More importantly, IL-17A and TNF-α individually strengthen the IL-22-induced IL-20 production. In line with this, in lesional skin of psoriasis patients, highly elevated IL-20 levels correlated less strongly with IL-17A and TNF-α than with IL-22. This study demonstrates a novel type of pathogenetic cascade that is important for the generation of the psoriasis-typical epidermis alterations and that constitutes of a T-cell mediator that induces a tissue cell as an amplifier of its own action.

P053

The epidermal alterations in psoriasis are mediated by IL-22, but not by other T-cell mediators including IL-17 or IFN-γ, and are amplified by TNF-α

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Psoriasis is a common chronic skin disease with a largely unknown pathogenesis. We demonstrate here that interleukin (IL)-22 is a key mediator of the epidermal alterations observed in this disorder. First, IL-22 was highly expressed in affected skin, and systemic IL-22 levels correlated with disease severity. Second, transgenic over-expression of IL-22 in mice resulted in psoriasis-like skin alterations including acanthosis and hypo granularity. Third, keratinocytes, but not skin fibroblasts, endothelial cells, melanocytes, or sub epithelial adipocytes were responsive to IL-22.In these cells, IL-22 enhanced the expression of antimicrobial proteins and inhibited the expression of proteins involved in the keratinocyte terminal differentiation. In line with its differentiation-altering effect, IL-22 caused psoriasis-like morphological changes in a three-dimensional human epidermis model, an effect that was not shared by interferon-γ or IL-17. Interestingly, the IL-22 affect on differentiation-regulating genes was dependent on STAT3, whose expression was simultaneously up regulated by IL-22. Tumor necrosis factor (TNF) -α amplifies some psoriasis-relevant IL-22 effects, which may be due to its enhancement of the expression of IL-22 receptor pathway elements, including IL-22R1 and STAT3. This study suggests that therapeutic targeting of the IL-22/IL-22 receptor system would be a promising therapeutic option for psoriasis that, compared to current anti-TNF-α therapy, would be of advantage by lacking immunosuppression.

P054

Wound healing in epidermolysis bullosa

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In many forms of EB, wound healing is severely impaired, often leading to chronic wounds and atrophic scars. Additionally, recessive dystrophic EB of the Hallopeau-Siemens type often involves contractures leading to the loss of fingers. Therefore, we expect genes mutated in EB also to play a role in wound healing. We performed *in vitro* wound healing assays with primary wt fibroblasts and RDEB fibroblasts from scarring and non scarring skin. Thereby we found that scratch closure in RDEB fibroblasts from scar tissue and non scarring tissue occurred much later than in wt fibroblasts. By real time PCR we also showed that expression levels of FGF7 and type 1 collagen were slightly higher in scratched than in untreated wt fibroblasts. Furthermore, we found that expression of type 7 collagen was elevated in RDEB compared to wt fibroblasts, while collagen 7 expressions in RDEB fibroblasts from scar tissue and non-scarring tissue were similar. By a genome wide study of gene expression in scarring and non scarring skin of EB patients and healthy persons by cDNA microarrays, we will identify genes whose expression significantly differs between the studied groups. We are also establishing a 3D *in vitro* wound healing assay to study the influence of the differentially expressed genes on wound healing in EB and the effect of various substances (cytokines and/or pharmaceuticals) on EB wounds in order to use them for the treatment of EB patients.

P055

Inversed expression pattern of pro- and anti-apoptotic proteins in the psoriatic plaques after infliximab treatment

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Aim: It has been recently shown that infliximab induces a caspase-independent programmed cell death of psoriatic keratinocytes *in vivo*. The aim of the present study was the further investigation of the underlying mechanism of keratinocyte apoptosis in psoriasis after TNF α blockage by infliximab.

Methods: A group of 14 patients were treated with infliximab (5 mg kg^{-1} at weeks 0, 2 and 6) for moderate-to-severe plaque-type psoriasis. Serial skin biopsies of uninvolved and of lesional psoriatic skin were taken at baseline and 5, 14 and 21 days after treatment. The expression of the proapoptotic proteins P53, apoptosis inducing factor (AIF) and Bax, as well as the antiapoptotic proteins Bcl-2, BclXL and NF-κB was analyzed by means of immunohistochemistry and image analysis.

Results: The treatment with infliximab caused a significant up regulation on the expression of the proapoptotic proteins P53, AIF and Bax in the psoriatic keratinocytes of the target plaques. Statistically significant up regulation was marked 5 days after treatment for P53, while for AIF and Bax 21 and 14 days later respectively. In addition, down regulation of the anti-apoptotic proteins Bcl-2, BclXL and NF-κB was observed after the treatment. Bcl-2 and NF-κB were significantly down regulated 14 days after the first infusion whereas BclXL 21 days later.

Conclusion: The results of this study document an inversed expression pattern of pro- and anti-apoptotic proteins in the psoriatic epidermis after infliximab treatment with a preponderance of proapoptotic proteins. This might indicate the involvement of the intrinsic, mitochondrial pathway of apoptosis in the keratinocyte cell death observed.

P056

Expression of hair follicle stem cell markers in Cylindrospiradenomas

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Cylindromas and Spiradenomas are benign skin tumors that can develop sporadically or at multiple sites as a result of an autosomal, dominantly inherited condition called Brooke-Spiegler Syndrome (BSS). BSS is associated with mutations in the CYLD gene. CYLD encodes a deubiquitinase that exerts an inhibitory effect on NF-κB signaling resulting in an anti-inflammatory and anti-proliferative effect. Cylindromas and Spiradenomas often appear as hybrid tumors (Cylindrospiradenoma) and share several histological traits suggesting their differentiation from the same pluripotent progenitor cell type. Because the lesions only arise in hair-bearing regions of the skin we asked whether Cylindrospiradenomas are derived from hair follicle stem cells (HFSC). Sections of Cylindrospiradenomas were analyzed by immunohistochemical techniques for expression of HFSC and hair follicle outer root sheath (ORS) markers. The ORS marker β1 Integrin was expressed at high levels throughout tumor nodules whereas the HFSC marker Keratin 15 (K15) was confined to cells surrounding duct-like formations within the neoplasms. Interestingly, the HFSC marker CD200 was also widely expressed in Cylindrospiradenomas. In contrast to β1 Integrin however, CD200 signal was also found in K15 positive cells lining the tubular structures in the tumors. CD200 acts as a signal against autoimmune attacks and confers immune privilege status to HFSC. It may fulfill a similar immunosuppressive function in HFSC-like Cylindrospiradenoma cells and enable them to maintain their proliferative and multi potent properties. The effects of anti-proliferative and anti-inflammatory substances on Cylindro(spiradeno)mas will be tested in an organ culture system that we have established and that allows us to maintain these tumors in culture for up to 6 days.

P057

The influence of loss-of-function mutations in the filaggrin gene on the recovery rate and the job continuation in patients with occupational hand eczema

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Genetic factors play an important role in the development of hand eczema. One of the known genetic risk factors are loss-of-function mutations in the filaggrin gene (FLG) which result in reduced production of a key epidermal protein filaggrin. Little is known about the impact of FLG null alleles on the course of chronic irritant contact dermatitis (CICD).The goal of our prospective cohort study was to investigate the influence of FLG null alleles with special regard to the percentage of those who recovered and were able to return to their same occupation in patients who were hospitalized at our clinic for treatment of hand eczema. For this investigation, a cohort of 197 patients with occupational CICD were genotyped for FLG null alleles R501X and 2282del4 and followed-up for one year after discharge. Patients were employed in one of the following occupational categories (I) healthcare, (II) metal work/construction, (III)hair dressing/beauticians, (IV) food/catering and (V) janitorial services. The statistical evaluation was conducted using the chi-square and the Fishers exact test where appropriate. Results were considered significant at $P \leq .05$.Our study comprised of 93 (47.2%) atopic individuals (AI) and 104 non atopic individuals (NAI). Overall, 24 patients showed a mutation in the FLG alleles R501X or2284del4 (14 AI and 10 NAI). Results revealed that 25.4% of all discharged patients could not return to their occupations. In carriers of the FLG null allele, the risk of abandoning their profession was significantly increased (45.8% vs. 22.5%; or 2.9; 95% CI: 1.2–7.0; $P = .014$). The prevalence of moderate and severe skin symptoms was higher in patients with FLG null allele 2282del4 compared to non carriers (47.1%vs. 21.4%, $P = .032$). In contrast, no significant difference was observed with regard to the recovery rate and job continuation when comparing AI and NAI ($P = .43$), nor when contrasting the combination of atopy and filaggrin mutations with the differences of FLG alone ($P = .69$).In conclusion, our data showed that patients suffering from hand eczema and having FLG mutations are more resistant to therapeutic approaches, resulting in lower rates of recovery and job continuation. Thus, early stage identification of individuals with FLG mutations may result in additional emphasis to this risk population in terms of specific preventive intervention and dermatological guidance.

P058

Waardenburg syndrome type I with heterochromia iridis and circumscribed hypo pigmentation of the skin

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We report a 3-year-old girl with the autosomal dominant inherited Waardenburg syndrome type I showing circumscribed hypo pigmentation of the skin, heterochromiairidis, sensorineural deafness and dental aberrations. Clinical diagnosis was confirmed by the identification of an underlying missense mutation (C811T) in thePAX3 gene. An early diagnosis of Waardenburg syndrome among children with pigment anomalies enables a successful interdisciplinary medical care.

P059

Filaggrin deficiency results in a dose-dependent impairment of the epidermal barrier in ichthyosis vulgaris

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Although it is generally presumed that filaggrin (FLG) deficiency accounts for abnormal permeability barrier function in ichthyosis vulgaris (IV), there is little direct evidence for this link in humans, and the mechanisms are unknown. In order to assess the relationship between FLG mutations and the epidermal permeability barrier, we assessed epidermal function and structure in 21 IV patients, 13 heterozygotes (+/–) and 8 homozygotes or compound heterozygotes ($^{-/-}$), and 20 age and gender-matched controls. The presence of single- or double-allele mutations dose-dependently correlated with decreased FLG protein expression, decreased SC hydration, and an increase in epidermal hyperplasia and number of stratum corneum (SC) cell layers. However, FLG-deficient corneocytes showed normal keratin aggregation, normal cornified lipid envelopes and only minor cornified envelope fragility upon exposure to a strong detergent and ultrasound. Instead, IV patient skin exhibited a FLG dose-dependent increase in TEWL, a delay in barrier recovery following barrier disruption, intercellular lanthanum leakage and impaired secretion/processing of lamellar body-derived extracellular lipid bilayers, consistent with a primarily extracellular barrier defect. In addition to a decrease in SC hydration and an increase in surface pH in FLG allele mutant SC as compared to controls, decreased downstream production of FLG-deficient scales resulted in decreased serine protease activity, and persistence of corneodesmosomes into the outer SC, attributable to impaired secretion of lamellar body-derived hydrolases. These results provide new insight into the pathogenesis of the epidermal permeability barrier dysfunction of FLG-deficient epidermis.

P060

''A New Disease is born'': the H Syndrome - an autosomal recessive genodermatosis with systemic manifestations

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The H syndrome is a recently described autosomal recessive genodermatosis with systemic manifestations. The name derives from its major clinical and laboratory findings, which comprise cutaneous hyper pigmentation and hypertrichosis, hepatosplenomegaly, heart anomalies, hearing loss, hypogonadism, hyperglycemia/diabetes mellitus, low height, hallux valgus and fixed flexion contractures of the toe and proximal interphalangeal joints. Involved skin has typical histological findings, which include a mainly histiocytic dermal and subcutaneous infiltrate, admixed with mast cells and plasma cells. This infiltrate is later replaced by fibrosis with thickened, fragmented and partially calcified elastic fibers and psammoma bodies. Homozygosity mapping and further sequencing revealed various mutations (missense, compound and deletion) in the SLC29A3 gene, which encodes the widely expressed equilibrative nucleoside transporter; hENT3, that localizes to the mitochondria. According to our knowledge this is the first description of a disease caused by mutations in nucleoside transporters, and it highlights their potential role in maintaining normal skin and hair homeostasis, inviting further studies to better lineate the role of these transporters in the skin.

P061

Marie Unna hereditary hypotrichosis caused by a novel mutation in the human hair less transcript

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Recently the causes for various forms of hypotrichosis and atrichia have been identified, increasing our understanding of the pathways involved in hair cycling and morphogenesis. Loss-of-function mutations of an inhibitory upstream ORF in the human hairless transcript were found as the cause for autosomal dominant Marie Unna hereditary hypotrichosis. At present only two studies from China identified several pathogenic mutations. We ascertained a Jewish Ashkenazi family with hypotrichosis simplex of the Marie Unna type in a mother and her two children. Sequencing of the U2HR in the 5′ UTR of the hairless gene resulted in the identification of a novel heterozygous missense mutation c.74 C > T resulting in the amino acid change p.P25L. This finding extends the mutations spectrum of U2HR, and emphasizes its major role in hair growth.

P062

Acral peeling skin syndrome resembles epidermolysis bullosa simplex: novel and recurrent mutations in the TGM5 gene

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Acral peeling skin syndrome is considered to be a rare autosomal recessive genodermatosis in the large group of disorders of cornification, which is characterized by peeling of the skin of hands and feet. So far, two missense mutations in the transglutaminase 5 gene, TGM5, have been described in patients. Here, we report acral peeling skin syndrome as an important differential diagnosis for epidermolysis bullosa simplex (EBS), since in our cohort of 83 patients suspected to have this epidermolysis bullosa form, 11% of the individuals carried transglutaminase 5 mutations. The initial assumption of EBS was understandable for several reasons: the presence of blisters mainly in infants, the fact that no tissue separation was observed in immunfluorescence mapping and the vertical transmission of a milder form of the disease, reported by many of the patients. The TGM5 mutation p.G113C was identified in nine unrelated patients, suggesting that it is a recurrent mutation in the Central and Northern European population. Further, we disclosed the novel mutation p.W255R and the new silent polymorphism p.Y220Y in the TGM5 gene. The mutation is located close to the active site of transglutaminase 5 and is predicted to affect enzyme activity. In the skin *in vivo*, the staining patterns of filaggrin, loricrin and involucrin were perturbed, disclosing functional consequences of the transglutaminase 5 mutations in the skin. In addition to their diagnostic relevance, these observations provide novel data on the role of transglutaminase 5 in the skin and further insight into the path physiology of acral peeling skin syndrome.

P063

Defects of the Kindler syndrome protein kindlin-1 cause dermal fibrosis: the role of inflammatory processes

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The Kindler syndrome (KS) protein kindlin-1 is an essential component of the integrin-signaling platforms within epidermal basal keratinocytes. Loss of kindlin-1functions leads to abnormalities of keratinocyte adhesion and motility and to skin blistering and progressive poikiloderma as clinical symptoms in KS. Dermal fibrosis develops with age and leads to scleroderma-like skin changes, particularly in the extremities. In this study we addressed the pathogenesis of dermal changes in the KS and investigated the expression of profibrotic molecules in the skin of 11 patients *in vivo*, and in KS keratinocytes and fibroblasts *in vitro*. In all KS skin samples the dermis was infiltrated by CD68 positive macrophages. The amounts of IL-6, TGF-β1, CTGF and α -smooth muscle actin were increased, and substantial deposition of tenascin C was found below the dermal-epidermal junction, suggesting persisting inflammatory processes and ongoing tissue regeneration, even in the absence of clinical blisters. Correspondingly, primary fibroblasts isolated from three different individuals with KS exhibited increased production of α -smooth muscle actin and collagen I, implicating an activated fibroblast phenotype, similarly to fibrotic conditions. The direct role of keratinocytes in the initiation of fibrosis was revealed by cDNA and protein expression arrays. Cytokine and growth factor expression profiling demonstrated that KS keratinocytes expressed higher levels of IL-6, IL-20 and IL-24 than control cells, suggesting that keratinocytes influence the development of fibrosisin a paracrine manner and via macrophages. We conclude that in KS skin an intra epidermal defect, loss of kindlin-1, perturbs epithelial-mesenchymal interactions and induces progressive dermal fibrosis through persistent inflammatory processes and secretion of profibrotic factors.

P064

Expression of LEKTI and normal SPINK5 genotype rule out allelic identity of Peeling skin syndrome type B and Netherton syndrome

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Peeling skin syndrome (PSS) type B (MIM 270300) is an autosomal recessive disorder of cornification that is characterized by lifelong patchy peeling of the entire skin. It is associated with severe pruritus, atopic diseases and frequent *Staphylococcus aureus* skin infections, differentiated from acral PSS that is due to mutations in TGM5, and PSS type A that represents a generalized PSS form without further clinical symptoms. Recent literature suggested that PSS type B may represent an allelic variant of Netherton syndrome (NS) that is due to recessive mutations in SPINK5 and resultant deficiency of LEKTI. We investigated a group of four affected children who suffered from superficial scaling since the second day of life, pruritus and atopic diseases. As such the phenotype resembled that of NS; however there were no hair shaft anomalies, i.e. notrichorrhexis invaginata. Histological analyses of the skin showed psoriasis form dermatitis and detachment of the stratum corneum similar to NS. Ultra structural analyses revealed a week lateral adhesion of corneocytes. We performed antigen mapping of LEKTI that - in contrast to NS - showed a normal or enhanced expression. Moreover, complete sequencing analysis of SPINK5 did not show any mutations. We conclude that PSS type B represents a genetically distinct disease entity different from Netherton syndrome.

P065

Distribution of mitochondrial DNA deletions in Csbm/m and Csa$^{-/-}$ mice of different ages

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Genotoxic stress can lead to cancer or aging. Defects in the repair mechanism nucleotide excision repair (NER) either lead to skin tumors in Xeroderma pigmentosum (XP) or premature aging and neuro degeneration in Cockayne syndrome (CS). Aging-associated reduction of subcutaneous fat is a hallmark of physiological aging, premature aging of CS patients as well as many other progeroid syndromes. Accumulated mutations of mitochondrial (mt) DNA are also linked to chronological aging, premature skin aging and neuro degeneration. Less is known about the age dependent distribution of mtDNA large scale deletions in multiple organs of prematurely aging mice. We investigated spleen, liver, skin (epidermis, dermis and subcutaneous tissue), quadriceps muscle, heart and brain of mice of different age groups and different genotypes (Csbm/m, Xpa$^{-/-}$ and wild type) for the relative amount of large mitochondrial deletions (mtD17 and mtD1). We found an age dependent increase of mtD17 in many organs in all mice. Csbm/m mice accumulated more mtDNA deletions than Xpa$^{-/-}$ and wild type mice of the same age group. For further investigation we micro dissected subcutaneous fat of Csa$^{-/-}$ and Csbm/m, Xpa$^{-/-}$ and wild type mice of different age groups and analyzed the relative amount ofmtD17 and the mtD1 deletion. Aged Csa$^{-/-}$ and Csbm/m mice show a strong increase of mtD17 and mtD1 in age-dependently reduced subcutaneous fat tissue which was not observed in other DNA repair deficient mice that do not show prematurely reduced subcutaneous fat. MtDNA deletions were increased in the epidermis and dermis of these animals but at much lower levels compared to subcutaneous fat. Sequence analysis and restriction enzyme digest confirmed the identity of PCR results. These data show that mtDNA mutations age-dependently increase in practically all tissues of progeroid animals with the highest levels in subcutaneous fat, indicating that these mutations may be involved in age dependent loss of subcutaneous tissue.

P066

Connecting base excision repair (BER) and nucleotide excision repair (NER) within mitochondria: Cockayne syndrome A and B interact in complexes with mitochondrial (mt) DNA repair associated proteins 8-oxo-guanosineglycosylase (hOGG)-1 as well as mt single strand binding protein (SSBP)-1

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DNA damage induced by ultraviolet (UV)-B radiation predominantly gets repaired By the nucleotide excision repair (NER) mechanism, while UV-A induced oxidative DNA damage is preferentially repaired by base excision repair (BER). Defective NER either causes skin cancer prone Xeroderma pigmentosum (XP) or premature aging in Cockayne syndrome (CS).While mitochondrial BER is well characterized; mitochondria are thought to be free of NER. Mutations of mitochondrial (mt) DNA have been linked to aging. We previously showed localization of CSA and CSB proteins in mitochondria, their enrichment upon oxidative stress, premature induction of oxidatively induced mtDNA mutations in CSA and CSB cells as well as binding of CSA and CSB proteins to mtDNA. However, it has been unclear if CSA or CSB interact with other mitochondrial proteins.

We provide evidence that CSA and CSB are associated to complexes of the DNA repair associated mitochondrial single strand binding protein (mtSSBP)-1 and the BER-associated 8-oxo-guanosine glycosylase (hOGG)-1 in oxidatively stressed mitochondria by co-immunoprecipitation. These data present a link between mtCSA/CSB proteins and protection from large scale deletions of the mtDNA via a previously unreported interaction of two hitherto separate repair mechanisms NER and BER.

P067

The clinico-neurological and cerebro-morphological fingerprint of xeroderma pigmentosum

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Clinical symptoms of the rare autosomal recessive disorder xeroderma pigmentosum (XP) comprise pathological skin symptoms; furthermore, depending on XP subtype, various neurological abnormalities have been reported. We present neurological alterations and brain imaging data of 14 patients of seven XP subtypes. Brain imaging investigation included conventional MRI scans and multiparametric MRI(N = 7) in order to assess the cerebral morphology *in vivo* at a macrostructural and microstructural level in comparison to controls, including volumetric measurements, MR spectroscopy (1H MRS), and diffusion tensor imaging (DTI). Clinical hallmarks were a spinocerebellar ataxia, pyramidal tract signs, and mild cognitive deficits. The pattern both for grey matter and for white matter alterations was heterogeneous among the group. It could be demonstrated that three patients showed a marked relative hippocampal volume reduction, but the patients were not different from controls in the volumetric measurements of hippocampal and whole brain volumes at group level. However, it could be demonstrated by 1H MRS that the hippocampal formation was metabolically altered. The most prominent feature was an affection of the white matter of the brain, as assessed by DTI, with volume and directionality reductions of the fiber projections involving both craniocaudal fibers such as the pyramidal tracts or thalamic fibers and interhemispheric connections such as the corpus callosum. In summary, our data are in support of XP-associated patho anatomy of the brain with mainly widespread white matter alterations. In contrast to the skin abnormalities in XP which depend in extent on sun exposure, the neurological abnormalities show a distinct pattern of vulnerability without an obvious trigger mechanism.

P068

Deletion of LCE3C and LCE3B genes at PSORS4 contributes to susceptibility to psoriasis vulgaris but not to psoriatic arthritis in German patients

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PSORS4 is a susceptibility locus for psoriasis vulgaris (PsV), a common inflammatory, hyper proliferative skin disorder. Recently, a deletion of two late cornified envelope (LCE) genes within epidermal differentiation complex on chromosome 1 was shown to be associated in 1,426 PsV patients, suggesting compromised barrier function in deletion carriers. In order to investigate whether this variant also predisposes to psoriasis vulgaris in German patients and whether it is also a risk factor for psoriatic arthritis (PsA), we genotyped this deletion and three SNPs in strong linkage disequilibrium with it in large case-control-cohorts of 1354 PsV patients, 650 PsA patients and 937 control individuals of German origin. While association to the LCE deletion was significantly associated to PsV (2 = 17.44, $P = 2.97^{*}10–5$, OR [95% CI] = 1.31 [1.15–1.48]), the frequency of the deletion did not significantly differ between PsA patients and controls (65.0% vs. 65.5%).

This is the first non-HLA risk factor predisposing only to skin-type of psoriasis supporting the concept of partially overlapping, but different etiological factors underlying skin and joint manifestations.

P069

Absence of Merkel Cell Polyoma Virus in Melanoma

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Recently, Merkel Cell Polyoma Virus (MCPyV) has been described in 80% of Merkel cell carcinomas (MCC). Similarly to MCC, melanoma incidence is increased in immuno-suppressed patients, and retrovirus-like particles have been identified inhuman melanoma using electron microscopy. We hypothesized that MCPYV may play a role in melanoma development as well. We selected 95 archival, paraffin-embedded melanomas and 8 MCCs. The cohort contained 47 superficial spreading melanomas, 15 nodular melanomas, 8 lentigomaligna melanomas, 19 acral melanoma melanomas, and 6 unclassified cutaneous melanomas. The median age of the patients was 67 (range 37–94). DNA was obtained from micro-dissected tissue and amplified with PCR primer sets specific for the MCPyV large T antigen locus (LT1 and LT3), and for the VP1 gene. As a positive control we used a Merkel cell carcinoma, tested positive with all three primer sets for MCPyV before. To determine the presence of amplifiable DNA in all melanoma samples, BRAF exon 15 was amplified. PCR products were separated on 1.5% agarose gels and visualized with Gel Red. None of the 95 melanoma samples did show LT1, LT3, or VP1 fragment amplification. DNA from a MCC run in parallel to the melanoma samples as a positive control produced a clearly visible band. PCR using BRAF exon 15 primers did proof the presence of amplifiable DNA in all 95 melanoma samples. Our study found no LT1, LT3 and VP1-PCR products in 95 human cutaneous melanomas. This is in accordance with a study that found no MCPyV innon-UV-associated primary melanomas of mucous membranes. In conclusion, there is no evidence that MCPyV infection and genome integration occurs in melanoma, o rat least not at a relevant frequency. However this finding does not exclude the possibility that other still unknown types of polyoma viruses are associated with melanoma.

P070

Functional correction of recessive dystrophic epidermolysis bullosa by 3′trans-splicing of COL7A1

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Functional defects in type VII collagen, caused by premature termination codons on both alleles of the COL7A1 gene, are responsible for the severe autosomal recessive types of dystrophic epidermolysis bullosa (RDEB). In these phenotypes type VII collagen is unable to form stable anchoring fibrils at the dermal-epidermal junction (DEJ) of the skin. Most gene therapy efforts for DEB are presently focused on the transfer of the full-length wild type COL7A1 cDNA into affected cells. However, the clinical application in the human system is hampered due to both the size and the highly repeated nature of the cDNA sequence. Therefore we use trans-splicing to reduce the size of the COL7A1 transcript for a gene therapy approach. Trans-splicing exchanges parts of the coding sequence of the endogenous target transcript by the wild type coding sequence, which is exogenously delivered by a repair molecule called pre-trans-splicing molecule (PTM).We were able to correct the RDEB phenotype in primary keratinocytes from a RDEB patient, carrying two nonsense mutations in COL7A1 exons 14 and 106 that provoke type VII collagen-deficiency. Retroviral transduction of these keratinocytes with a 3′PTM, encoding COL7A1 wild type exons 65-118, resulted in correction of full-length type VII collagen expression. Skin equivalents reconstructed with the corrected RDEB keratinocytes *in vitro* showed deposition of type VII collagen at the basement membrane zone of the DEJ, where it assembled into anchoring fibril-like structures. To attest to the full phenotypic and functional reversion of the trans-splicing corrected RDEB keratinocytes *in vivo*, patches of skin equivalents cultured from these keratinocytes were grafted onto immunodeficient mice. Histological and immunohistological analysis of five-week old specimens of the grafted tissue showed no blistering and strong labeling of human type VII collagen between the dermis and the epidermis. Localization of type VII collagen was restricted to the basement membrane, with no expression in the suprabasal cells layers, consistent with the idea that the recombinant type VII collagen is tightly regulated *in vivo*. In this work we demonstrated that 3′ trans-splicing within the endogenous COL7A1 gene generates tissue-specific stable expression of human type VII collagen *in vitro* and *in vivo*. Thus, 3′ trans-splicing may be suitable for an *ex vivo* gene therapy approach for treatment of DEB.

P071

Expression pattern of kallikrein-related peptidase 12 (KLK12) in human skin

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Human kallikrein 12 (KLK12) is a member of the human tissue kallikrein family, a group of 15 secreted serine proteases which are found on chromosome 19q13.4.Some KLKs are described as potent cancer biomarkers, but for most the physiological function is unknown. KLK12 was previously shown to be expressed in a variety of tissues including salivary glands, bone marrow, stomach, uterus, etc. but investigations failed to detect KLK12 mRNA as well as protein expression in human skin. In this current work we characterized the protein and mRNA pattern of KLK12 by semi-quantitative real time PCR analysis, RT-PCR and Western blotting in human skin samples. For the first time we identified all three splicing variants of KLK12 in whole skin samples whereas PCR analysis of mRNA isolated from epidermal samples did not show expression of transcript variant 1 (NM_019598.2). Western blotting of proteins isolated from whole skin revealed that KLK12 was hardly detectable suggesting a minor role of this serine protease in healthy skin. Nevertheless, further investigations towards the expression pattern of KLK12 in various pathological skin samples such as basal cell carcinoma, squamous cell carcinoma or morbus bowen has to be performed to give better insights in possible function of KLK12 in human skin.

P072

Staphylococcal α-toxin is a strong inducer of IL-17 in chronic inflammatory skin diseases via IL-1R

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Background: The majority of patients with atopic dermatitis (AD) and psoriasis is colonized with *Staphylococcus aureus* (*S. aureus*). In AD, *S. aureus* colonization is positively correlated with the severity of eczema. A subgroup of 34% AD patients has been shown to be colonized with α-toxin producing *S. aureus*. Recent studies show that an altered IL-17 immune response results in diseases associated with chronic skin infections.

Aims: We sought to elucidate the effect of sublytic α-toxin concentrations on IL-17production in (i) peripheral blood mononuclear cells (PBMCs), T cells, Th1, Th2 andTH17 cells from buffy coats and in (ii) PBMCs and T cells from AD and psoriasis patients compared to healthy controls.

Methods: IL-17 induction was investigated in PBMCs, T cells, Th1, Th2 and Th17cells as well as in Th1, Th2 and TH17 T cell clones from reactive atopy patch tests from AD skin upon α-toxin stimulation in a time and dose dependent manner on protein and mRNA level. For blocking experiments anti-IL-1R anti-IL-6 were added, respectively.

Results: Sublytic α-toxin concentrations induced IL-17 in PBMCs, isolated CD4+ T cells, Th17 cells and Th17 T cell clones, whereas no effect was observed in Th1 andTh2 T cells and clones. IL-17 was directly induced by stimulation with α-toxin as well as dependent on IL-1 as shown by stimulation of T cells with supernatants from α-toxin stimulated purified monocytes and blocking experiments with anti-IL-1R.Moreover, we could show an enhanced IL-17 secretion upon stimulation with α-toxin in PBMCs and T cells from AD patients as well as psoriasis patients compared to healthy controls. By trend, AD patients secreted more IL-17 compared to psoriasis.

Conclusion: Our data clearly show that sublytic α-toxin concentrations induce IL-17directly and in an IL-1 dependent manner. AD and psoriasis patients secreted significantly more IL-17 upon stimulation with α-toxin compared to healthy controls. This could partially explain why skin colonization with *S. aureus* can contribute to chronic skin inflammation.

P073

The novel T-cell cytokine IL-31 modulates the functional activity of eosinophil granulocytes in humans

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Background: The novel T-cell derived cytokine IL-31 has been described for the induction of severe pruritus and dermatitis in transgenic mice. In humans we could recently show that IL-31 levels are increased in atopic dermatitis and correlate with disease severity.

Aim: In atopic dermatitis, eosinophil granulocytes represent key target effector cells, thus we thought to investigate the functional role of IL-31 on eosinophils.

Methods: Peripheral blood eosinophils were isolated by CD16 negative selection (purity >98%). IL-31 receptor A expression was analyzed with FACS analysis and Light-Cycler PCR, Ca2+ mobilization was determined fluorometrically; super oxidanion release was analyzed by lucigenin-dependent chemiluminescence; apoptosis, CD69 surface expression and CXCL8 release with FACS analysis; and STAT-3phosphorylation with Western Blot.

Results: Highly purified peripheral blood eosinophils expressed the IL-31 receptor A.IL-31 stimulation led to a significant increase of Ca2+ influx, a significant release of superoxide anions and CXCL8 of eosinophils. Further, IL-31 increased the surface expression of CD69 on peripheral blood eosinophils, whereas eosinophil apoptosis was not modified. Finally, IL-31 stimulation led to a phosphorylation of the downstream signaling molecule STAT-3.

Conclusion: Together, these data give first evidence for a functional role of IL-31 on peripheral blood eosinophils, thus revealing novel implications for IL-31 action.

P074

Disclosure of the culprits: A novel macrophage activation pattern and iron drive the non-healing state of chronic venous leg ulcers

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Chronic venous leg ulcers (CVU) fail to progress through a normal pattern of wound repair, but instead remain in a chronic inflammatory state with little signs of healing. We have earlier suggested that the persistent infiltration of macrophages (Mϕ) in conjunction with iron derived from extra vasated erythrocytes contribute to a hostilepro-oxidant microenvironment. However, the underlying mechanisms and the final *in vivo* proof are still missing. We here addressed the questions (1) whether a specific pro-inflammatory Mϕ activation phenotype with excessive TNFα prevails in CVU, (2) whether iron and distinct reactive oxygen species (ROS) persistently activate such apro-inflammatory macrophage subset perpetuating the non-healing state and (3) whether chelation of iron or neutralization of TNFα may rescue Mϕ activation and impaired wound healing?

Notably, immunostaining of ulcer sections and FACS analysis of Mϕ isolated from 6CVU patients revealed a distinct activation phenotype with high expression of both classical, pro-inflammatory (CD18, CD59, IL-12, and TNFα at high levels) and alternative, anti-inflammatory (Arginase, Dectin-1, IL-10, CD206, at moderate levels) activation markers. By sharp contrast, a strong but transient classical Mϕ activation phenotype was observed in acute wounds at day 2, while an anti-inflammatory Mϕ activation pattern occurred at day 5 post wounding (each n=6). Mimicking the Fenton reaction *in vitro* by exposure of Mϕ derived from human peripheral blood mononuclear cells to a combination of iron and hydrogen peroxide (H2O2) with subsequent generation of hydroxyl radicals (OH.) led to the induction of an identical combined Mϕ activation pattern with high TNFα release. Prussian blue staining detected iron deposition both in CD163+ Mϕ and extracellularly in skin section from CVU patients. Interestingly, incubation of sections from CVU patients and acute wounds with the redox sensitive dye Dyhydrorhodamine (DHR) and specific ROS scavengers identified a persistent increase in the highly aggressive OH in the margins from CVU, whereas only a transient accumulation of H2O2 occurred in acute wounds.

To study the causal role of the iron-driven Fenton reaction for the Mϕ activation during wound healing *in vivo*, we established an iron-load mouse model by i.p.injection of iron-dextran preferentially phagocytosed by Mϕ. Interestingly, this model closely recapitulates major aspects of CVU with impaired wound healing, combined Mϕ activation with up-regulation of both classical (CD18, Ly6C, TNFα, CCR2) and alternative (IL-10, CD204, CD206, CD301) activation markers and marked increase in OH. Treatment of these mice with either the iron chelating Desferrioxamine or the TNFα antagonist Etanercept before wounding completely rescued the wound healing deficiency. These data very much suggest that the iron-driven Fenton reaction with the generation of OH is responsible for the combined Mϕ activation phenotype with high levels of TNFα. Thus, targeting activation of this newly identified Mϕ subset may prove beneficial for this difficult-to-treat condition.

P075 (V07)

The TLR4 ligands S100A8/A9 play a critical role in the development of auto reactive CD8+ T cells

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Autoimmunity results from a conflict of regulatory immune mechanisms controlling self-tolerance but the cellular and molecular patho mechanisms underlying the loss of immuno tolerance against self remain largely unknown. The regulation of the adaptive immune system is initiated by antigen-presenting cells (APC) that can activate naïve T cells in a MHC dependent fashion. In particular the interaction of the receptor CD40on APC with its ligand CD40L plays an important role during immune responses. Within the skin, transgenic (tg) over expression of CD40L in basal keratinocytes spontaneously leads to systemic autoimmunity as evidenced by auto antibodies, nephritis, protein urea, and autoimmune dermatitis, which can be adoptively transferred by injecting CD8+ T cells into naive recipient mice. However, the molecular mechanisms linking the local micro milieu and the systemic development of auto reactive CD8+ T cells in CD40L-induced systemic autoimmunity are poorly understood. Calcium-binding proteins of the S100 family such as S100A8 andS100A9 are damage associated molecular pattern molecules (DAMPs) highly up-regulated in various autoimmune disorders. Hence, we investigated the relevance of S100A8 and S100A9 for the development of functional auto reactive T cells and could show that local S100A8 and S100A9 production is essential for the induction of auto reactivity in CD8+ T cells and the development of systemic autoimmunity. This effect is mediated via TLR4 signaling and up-regulation of the transcription factorsRunx-1 and RORc in CD8+ T cells leading to increased IL-17 expression. Notably, S100A8 and S100A9 expression was increased in cutaneous lesional lupuserythematosus and enhanced concentrations of S100A8 and S100A9 were detectable in patient's serum. Strikingly, stimulation of CD8+ T cells from lupuserythematosus patients with S100A8 and S100A9 proteins resulted in a significant up-regulation of IL-17 expression indicating that S100A8 and S100A9 play also an important role during human MHC class I-mediated cellular autoimmunity. Together, these results present the first link between local expression of a DAMP-molecule and the development of systemic autoimmunity.

P076

α-Melanocyte stimulating hormone induced tolerogenic dendritic cells generate functional regulatory T cells *in vitro* and *in vivo*

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The neuro peptide α -melanocyte-stimulating hormone (α-MSH) is a potent immunomodulator that is able to induce immunosuppression and tolerance. In the skin, α-MSH is expressed by keratinocytes and released into the circulation. To elucidate the mechanisms underlying α-MSH-induced immunomodulation we investigated whether α-MSH affects dendritic cell (DC)-T cell communication since especially this interaction plays an important role in the induction and regulation of immune responses. DC stimulated with α-MSH showed a reduced expression of MHC class II and costimulatory molecules compared to non-stimulated controls but demonstrated an increased expression of CD205 a surface marker that has been previously associated with DC-mediated induction of CD4+CD25+ regulatory T cells. Additionally, DC stimulated with α-MSH secreted higher amounts of IL-10 further pointing to immunosuppressive DC functions. To investigate whether α-MSH stimulated DC were able to induce a regulatory phenotype in naive CD4+ T cells, bone marrow-derived DC were treated with α-MSH and co-cultured with CD4+ T cells. Interestingly, CD4+ T cells from co-cultures with α-MSH stimulated DC were anergic produced high levels of IL-10 and suppressed the proliferation of CD4+CD25- effector T cells *in vitro*. Moreover, CD4+ T cells co-cultured with α-MSH stimulated DC displayed enhanced expression of markers characteristic for regulatory T cells (Foxp3, Neuropilin-1, CTLA-4 and TGF-β) as evidenced by multi color flow cytometry and quantitative real time-PCR. To test whether CD4+ T cells co-cultured with α-MSH treated DC were suppressive *in vivo* T cells from co-cultures with PBS- as well as α-MSH stimulated DC were injected into mice previously sensitized to the contact allergen DNFB. Upon ear challenge with DNFB, mice that had been treated with CD4+ T cells co-cultured with PBS stimulated DC mounted a normal contact hypersensitivity response. Strikingly, injection of CD4+ T cells from co-cultures with α-MSH treated DC inhibited contact allergy. Moreover, upon adoptive transfer α-MSH stimulated DC were able to ameliorate on going psoriasis-like skin inflammation in mice topically treated with imiquimod via the *in vivo* induction of functional regulatory T cells. Together, these findings indicate that stimulation with α-MSH results in immunosuppressive DC functions as well as an enhanced capacity to induce functional CD4+CD25+ regulatory T cells *in vitro* as well as *in vivo*.

P077 (V06)

Cathelicidin antimicrobial peptide LL-37 enables uptake of dsDNA into human epidermal keratinocytes

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Antimicrobial peptides (AMPs) are effector molecules of cutaneous innate immunity and provide a first barrier of defence against microbial pathogens. In the mean time, an array of additional functions of AMPs has been characterized. Due to their multiple activities as regulators of adaptive immune responses and inflammation the term'alarmins' has been introduced to designate the different AMPs. In particular LL-37, one biological active peptide form of the cathelicidin AMP family, exerts additional functions in the innate defense system and is able to modulate adaptive immune responses. Furthermore, it was demonstrated that human cathelicidin LL-37 enables a response to self-DNA by plasmacytoid dendritic cells (pDC) and therefore may participate in the activation of psoriasis: Cathelicidin LL-37 converts non stimulatory self-DNA from apoptotic keratinocytes in psoriatic skin into a potent trigger of pDC activation by forming a LL-37/DNA complex. The uptake of the LL-37/DNA complex into pDCs follows the endocytic pathway and recently it was shown that LL-37 is also able to act in a similar manner to induce the uptake of self-RNA released by dying cells. Whether cathelicidin transports DNA into resident epithelial cells in psoriasis is unknown. In this study we confirm that cathelicidin is over expressed in keratinocytesin lesional skin in psoriasis. Western blot analyses demonstrate the presence of the4.5 kDa form of LL-37 in psoriatic plaques. *In vitro*, LL-37 is able to mediate the uptake of short dsDNA (300 bp to 1000 bp) into primary human epidermal keratinocytes (NHEK) as demonstrated by immunofluorescence and FACS. LL-37and dsDNA localized to the cytoplasmic and nuclear compartment as observed by cell compartment fractionation and subsequent dot blot analyses. In contrast to previous publications in other cell types such as pDCs the uptake of dsDNA andLL-37 into NHEK was not dependent on endosome formation as dsDNA uptake was not inhibited by chloroquine and bafilomycin. In summary we observed that cathelicidin LL-37 is able to deliver dsDNA into primary keratinocytes independent of the endocytic pathway. This mechanism may contribute to the 'alarmin' function of LL-37, whose expression is dysregulated in several skin diseases such as psoriasis, rosacea or chronic wounds.

P078 (V24)

The role of cytokines and the major histocompatibility complex locus H2s insusceptibility to experimental epidermolysis bullosa acquisita

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The immune system detects and eliminates foreign antigens to protect an organism from disease. Aberrant immune responses can result in autoimmune diseases: The generation of autoantibodies against type VII collagen, which can be traced back to self-reacting B and T lymphocytes, affects an integral part of the dermal-epidermal junction (DEJ): A local deposition of autoantibodies leads to epidermolysis bullosa acquisita (EBA), a severe blistering disease of the skin and mucous membranes. After establishing a murine active disease model for EBA, we can show different disease activities of mouse strains as SJL and BALB/c upon a single immunization with a recombinant fragment of murine type VII collagen (mCol7C). We examined draining lymph nodes by immunohistochemical staining, laser-microdissection and quantitative RT-PCR, serum samples by ELISA and skin samples by confocal laser-scanning microscopy to test our hypothesis of a Th1-polarization with subsequent formation of pathogenic IgG2b-autoantibodies insusceptible SJL mice and a shift towards Th2 in resistant mice of the BALB/c strain with generation of non-pathogenic IgG1-autoantibodies.Examined draining lymph nodes of susceptible SJL mice feature a significant increase in T cell proliferation (3-fold at maximum), type VII-collagen-specific plasma cell numbers (10-fold) and mRNA-expression ratios of Th1-/Th2-specific cytokines asIL-12/IL-4 (4.5-fold), IFN-γ/IL-4 (10-fold) and TNF-α/IL-4 (5-fold) compared to resistant mice of the BALB/c strain. In line with these observations, we find significantly elevated levels of type VII collagen-specific IgG2b in the serum of SJL mice (32-fold), which has previously been shown to be the most pathogenic antibody subclass because of its complement-fixing ability. Binding of IgG2b to the DEJ was also increased significantly in SJL mice compared to BALB/c mice (13-fold). To corroborate the dependency of EBA-pathogenesis on a Th1-/Th2-polarization, we recently added Th1-prone C57BL/6 mice to our studies. However, C57BL/6 mice turned out to be resistant, which is strongly suggestive of an additional pathogenic factor such as the genetically determined haplotype of the murine major histocompatibility complex locus H2: Resistance could be linked to H2d (BALB/c) and H2b (C57BL/6), whereas susceptibility is noticed in H2s mice (SJL).

P079

Modulation of experimental epidermolysis bullosa acquisita towards an improved phenotype by initiating the production of non-pathogenic autoantibodies

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Epidermolysis bullosa acquisita (EBA) is a severe autoimmune disease. It is characterized by subepidermal blisters induced by binding of autoantibodies against type VII collagen, an integral part of the dermal-epidermal junction (DEJ) in the skin. Immunization of SJL mice with recombinant murine type VII collagen (COL7c) and the adjuvant TiterMax results in production of autoantibodies of the IgG2 subclass. These autoantibodies lead to complement fixation at the skin, infiltration of neutrophils and the blistering phenotype. In a first experiment I wanted to analyse, if it is possible to induce the production of non-complement fixing autoantibodies of the IgG1 subclass which may have an ameliorating effect on the disease progression. I could show that it is possible to modulate the SJL mouse system with the use of the adjuvant Alum: Mice immunized with the antigen COL7c and a mixture of the adjuvants TiterMax and Alum show an isotype-shift to COL7c-specific IgG1 in the serum. The Alum-treated animals feature an increased IgG1 deposition at the DEJ and a reduced deposition of complement-fixing IgG2 autoantibodies. The Alum treatment prevents a clinical phenotype of EBA. Due to the fact that the Alum treatment leads to a production of non-pathogenic autoantibodies and can prevent a disease progression we asked, if the presence of these autoantibodies could avoid the induction of disease. Therefore mice were pre-treated either with PBS (control) or the antigen COL7c in a combination of the adjuvants TiterMax and Alum. After this pre-treatment the mice were immunized withCOL7c and TiterMax only. The PBS pre-treated group showed a normal disease pattern. Around ten percent of the body surface was affected with blisters, erosions and alopecia in week ten after disease induction. In contrast the Alum pre-treated group showed a significantly improved course of disease with a score of only one percent of affected skin. These results may form the basis for developing novel strategies to treat EBA.

P080

Parallel application of two new ELISA Systems: Anti-BP230 andanti-BP180-NC16A-4X provide the most sensitive serological diagnosis of bullous pemphigoid

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Bullous pemphigoid (BP) is an autoimmune sub epidermal blistering disease associated with autoantibodies to BP180 and BP230. In the present study, an already well established anti-BP180-NC16A-4X-ELISA was complemented by a newly developed anti-BP230-ELISA. A fragment of the C-terminal globular domain ofBP230 (amino acid residues 2326-2649) was expressed in *E. coli*, purified and used as solid phase in an ELISA for the determination of anti-BP230-antibodies. Sera from clinically and immuno pathologically characterized patients with BP (n = 118), pemphigoid gestationis (PG; n = 20), linear IgA dermatosis (LAD; n = 20), mixed arthritides (MA; n = 170), systemic lupus erythematodes (SLE; n = 56) as well as elderly patients with non-inflammatory skin diseases (EP; n = 101; age >70 years) and healthy blood donors (HBD; n = 483) were analyzed. Autoantibodies to BP230 were detected in 67 BP sera (sensitivity 56.8%), in one patient with PG and LAD, respectively, and in 2.7% of control subjects (3 MA, 5 SLE, 3 EP, 10 HBD; specificity97.3%). Additionally, the anti-BP180-NC16A-4X-ELISA was performed following the manufacturers instructions, which showed in accordance with previously published data 89.0% positive reactions in BP and 1.6% in controls (n = 754), respectively. Interestingly, anti-BP230 autoantibodies were identified in 38.5% (5/13) of anti-BP180-NC16A-4X ELISA-negative BP sera. Regarding the detection of circulating autoantibodies in BP, the new anti-BP230-ELISA provides increase insensitivity from 89.0% to 93.2% when used in parallel to theanti-BP180-NC16A-4X-ELISA. It is therefore recommended to apply both ELISA systems concurrently in serological diagnosis of BP.

P081

ELISA for determination of autoantibodies against envoplakin and periplakin in paraneoplastic pemphigus

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Paraneoplastic pemphigus (PNP) is an autoimmune blistering skin disease characterized by circulating IgG autoantibodies against desmosomal constituents. In PNP, indirect immunofluorescence microscopy (IIFM) on rat bladder sections is most widely used to search for circulating autoantibodies that are most commonly directed against the plakin proteins envoplakin and periplakin. A sensitive and specific detection system for PNP-specific autoantibodies is not yet available but needed to differentiate PNP patients from those with pemphigus vulgaris in whom no tumor screening is required. Therefore, we generated overlapping recombinant fragments spanning the full length of envoplakin and periplakin. Subsequently, reactivity of a small number of PNP sera with these fragments was analyzed by immunoblotting and ELISA. The 3 best performing ELISA were used to determine IgG antibodies in a large number of sera from patients with PNP (n = 31), pemphigus vulgaris (n = 30), and bullous pemphigoid (n = 50) as well as healthy volunteers (n =140). The results were compared with those obtained by immunoblotting of extract from cultured human keratinocytes. Immunoblotting with recombinant fragments of envo- and periplakin revealed that most sera contained antibodies against the N-termini of envoplakin and periplakin as well as against the C-terminus of envoplakin. By ELISA, reactivity against envoplakin (amino acids 1–481), envoplakin (amino acids 1626–2033), and periplakin (amino acids 1–324) were found in 25, 25, and 23 of 31 PNP sera, and in 1, 3, and 2 of 220 control sera. This resulted in sensitivities and specificities of 80.6% and 98.8% for envoplakin (amino acids 1-481), 80.6% and 96.3% for envoplakin(amino acids 1626–2033), and 74.2% and 97.5% for periplakin (amino acids 1–324), respectively. In conclusion, the novel ELISA based on the N-terminal fragment envoplakin (amino acids 1626–2033) showed the highest diagnostic accuracy to detect circulating autoantibodies in PNP and will be a helpful tool for the diagnosis of this disease.

P082

Optimization of the T-cell stimulation capacity of a dendritic-cell-based melanoma vaccine

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Tumor-antigen-loaded dendritic cells (DC) are promising tools as therapeutic vaccine for the treatment of malignant melanoma. Although immune responses, such as induction of tumor-antigen-specific T cells, were observed in many studies with DC vaccines, these did not correlate with clinical responses. Electroporation of monocyte-derived DC with RNA, to load them with antigen, or to manipulate their function, has become a widely used method, also in clinical applications. Our aim in this study was to optimize the T-cell stimulation capacity of DC. Therefore, we electroporated an optimized CD40L-encoding RNA (optCD40L) alone, or a combination of (non-optimized) CD40L-, CD70-, and constitutively active TLR4 (caTLR4)-encoding RNAs (TriMix, as described by K. Thielemans et al.) into mature or immature DC, respectively. In addition, these DC were electroporated with an RNA encoding a tumor antigen (Ag). We chose Melan A as a model-Ag, for its well-characterized and highly immunogenic HLA-A2-presented peptide EAAGIGILTV. The optCD40L transfection in mature DC resulted in enhanced expression of the maturation markers CD25, CD40, CCR7, CD70, and OX40L, and in secretion of the pro-inflammatory cytokines IL-12p70, IL-6, IL-8, and TNF-α. Simultaneously, the DC retained their capacity to migrate in a CCR7-dependent way. The DC's capacity to expand autologous T cells was analyzed by *in vitro* stimulation and subsequent HLA-A2-MelanA tetramer-staining in combination with phenotyping (CCR7/CD45RA) for T-cell function. The capability of the DC to induce expansion of T cells in a second and third round of stimulation was improved by the transfection of optCD40L RNA. The TriMix transfection in immature DC resulted in maturation of these DC, as shown by up-regulation of maturation markers, and a secretion of IL-12p70. However, the T-cell expansion capacity was less efficient compared to cytokine-cocktail-matured, Melan A-RNA-electroporated DC. We believe that these studies point the way to improved DC that will induce better and longer lasting immune responses in the vaccination against cancer.

P083

Examination of T-helper cell / DC cross-talk by the transfection of T cells with RNA coding for TCRs

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The goal of immunotherapy of cancer is to initiate an adaptive immune response against the tumor. But up to now, cancer immunotherapy has mainly focused on the generation of tumor-specific CD8+ T cells, even though CD4+ T-cell help is also required for a long-lasting cytotoxic T lymphocyte response. However, the exact mechanism by which CD4+ T cells license dendritic cells (DC) and thereby modulate the priming and expansion of CTLs is not yet fully understood. For this purpose, we transferred melanoma-antigen-specific TCRs by RNA- electroporation into CD4+ T cells and co-cultured them with peptide-loaded DC to elucidate T-cell / DC cross-talk. We either introduced MAGE-A3/HLA-DP4-specific or gp100/HLA-A2-specificTCR-RNA into CD4+ T cells, which mainly resulted in antigen-specific Th1 cytokine secretion after co-cultivation with peptide-loaded DC. Besides, phenotypic changes were assessed in a time-dependent manner. We detected a clear antigen-specific up-regulation of maturation markers like CD25, CD40, CD80, CD86, and CD70 on both immature and mature DC after 20 h of co-cultivation with T cells. In correlation, CD25 was antigen-specifically up-regulated as an activation marker onCD4+ T cells. The early activation marker CD69 was antigen-specifically up-regulated already after 2 h, while CD27- and CCR7-expression on the T cells remained almost constant during the stimulation. Moreover, T-cell activation was completely cell-cell contact dependent, while the maturation of the DC was in part mediated by soluble factors, as revealed by transwell experiments. These data indicate that DC / CD4+ T-cell cross-talk is a bidirectional process. Moreover, we currently examine the influence of pre-activation of CD4+ T cells on the antigen-specific cross-talk, and whether there is an antigen-specific cross-talk between DC and CD8+ T cells. Since TCR-transfected CD4+ T cells can induce DC maturation, they may be used to provide T-cell help to induce more efficient CD8+T-cell responses for the immunotherapy of cancer.

P084 (V01)

B7H-positive myeloid derived suppressor cells (MDSC): Cross-talk of novel phenotypes of MDSC and regulatory T cells (Tregs) during melanoma growth

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Myeloid derived suppressor cells (MDSC) comprise a phenotypically heterogeneous population of cells (CD11b+, Gr-1+), which can be found in tumor bearing mice and in patients with cancer. Though there are several reports suggesting that MDSC suppress the activity of T cells during tumor growth, the exact mechanism of their suppressive function is still unclear. To investigate the expression of suppressive molecules (B7H1, B7H3, B7H4) on MDSC during melanoma growth, we injected RET melanoma cells into C57BL/6 mice. After tumors had reached defined sizes, mice were sacrificed and single cell suspensions of tumors and spleens were prepared. FACS analysis revealed significant numbers of CD11b+Gr-1+ cells in the tumors and spleens from tumor-bearing mice but only CD11b+ Gr-1+ cells in tumors initially up-regulated the expression of B7H1, B7H3 and B7H4. However, these molecules were gradually down-regulated during tumor growth. To assess the 'cross-talk' of regulatory T cells (Treg) and MDSC, we examined the MDSC from CD25-depleted or non-depleted tumor bearing mice. After depletion of the CD4+CD25+Foxp3+ Tregs by antibody injection (PC61) *in vivo*, the expression of B7H1, B7H3 and B7H4 was significantly down-regulated on CD11b+ cells. When CD11b+ cells were isolated by MACS and co-cultivated with syngeneic CD4+ T cells and anti-CD3 antibodies, tumor-derived CD11b+ cells suppressed the proliferation of CD4+ T cells by NO production. In contrast, splenic CD11b+ cells from control mice did not suppress the proliferation of syngeneic CD4+ T cells. Our results suggest that only tumor infiltrating CD11b+ cells, which express B7H regulatory molecules in the presence of Treg in the tumor microenvironment, suppress T cell proliferation during melanoma growth.

P085

Differentiation of IL-17 secreting T cells is supported by human dermal fibroblasts via up-regulation of IL-23 production by dendritic cells

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TH17 cells are a recently identified helper T-cell (TC) subpopulation, which is characterized by secretion of interleukin (IL)-17A. These cells play an important role in autoimmunity and pathogen defence. Here, we show that fibroblasts support the differentiation of TH17 cells via up-regulation of IL-23 production by dendritic cells (DC). To trigger an effective TC-mediated immune response in the skin, upon antigen contact DC migrate into locally-draining lymph nodes where they present antigen to naïve TC and thereby induce their activation and differentiation. During their migration to secondary lymphoid organs, DC travel through the stromal microenvironment comprised of the extracellular matrix and stromal cells such as fibroblasts, macrophages and endothelial cells. Little is known about the interaction of DC with the stromal microenvironment. Recently, we have shown that DC interacts with dermal fibroblasts in inflamed skin both *in vivo* and *in vitro*. To study the effects of this interaction, monocyte-derived DC were partially matured by adding Lipopolysaccharide (DC-LPS) to imitate antigen contact in inflamed skin. Following, they were cocultured with dermal fibroblasts for 24h. We could demonstrate that LPS-stimulated DC activated fibroblasts via tumor necrosis factor (TNF) α and IL-1β. Consequently, activated fibroblasts produced prostaglandin (PG) E2 which in turn increased IL-23 production by DC compared to DC stimulated with LPS alone. Since IL-23 is an important factor in the differentiation of TH17 cells, we stimulated CD3+, CD4+, memory or naïve TC with either supernatants of DC-LPS-fibroblast-coculture or DC-LPS in the presence of anti-CD3/CD28-beads.Indeed, supernatants of DC-LPS-fibroblast-coculture significantly increased IL-17production of CD3+, CD4+ T cells or memory TC compared to TC stimulated with DC-LPS supernatant alone. In contrast, IL-17 was not elevated in naïve TC in presence of the cell culture supernatants. In summary, we were able to demonstrate that dermal fibroblasts regulate cytokine production of DC and thus are involved in the control of TC differentiation.

P086 (V18)

Mast cell committed progenitors traffic through blood, peripheral tissue and lymph - implication for the replenishment of mature mast cells due to inflammatory signals.

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Hematopoietic stem and progenitor cells (HSPCs) have been shown to re-circulate through blood, lymph, and extra medullary tissues and to give rise to tissue-resident myeloid cells, preferentially dendritic cells, upon inflammatory stimuli. Recently, mast cell-committed progenitors (MCPs) have been described among HSPCs in the bone marrow of adult mice. Here, we demonstrate thatLin-Kit+Sca-1-Ly6c-CD27-FcεRIα-β7-integrin+T1/ST2+ MCPs similarly traffic though blood, peripheral tissue, and lymph. We show that MCPs are detectible in blood and peripheral tissue, i.e. skin, and that mouse thoracic duct lymph contains MCPs that possess mast cell (MC) reconstitution capacity. FTY720 treatment, a potent sphingosine-1-phosphate (S1P) receptor antagonist, results in the depletion of MCPs from lymph indicating that the egress of MCPs from peripheral tissue into the lymph is a S1P receptor dependent mechanism. Prolonged topical application of the phorbolester phorbol 12-myristate 13-acetate (PMA) results in pronounced dermal MC accumulation. Systemic pertussis toxin (PTx) treatment markedly impaired the recruitment of MCPs to PMA treatment sites and reduced the number of accumulating mature MCs. Thus, we demonstrate that the migration of MCPs into peripheral tissue, i.e. the skin, requires the expression of functional Gzi-coupled receptors. In summary, our findings indicate that MCPs traffic through bone marrow, blood, periphery tissue and lymph under physiological conditions, and that MC accumulation due to inflammatory signals depends on the recruitment of MCPs and/or the inhibition of their egress from tissue sites. More detailed characterization of the regulation of MCP trafficking could lead to a better understanding of MC-mediated diseases and the development of novel treatment strategies.

P087

TCR-RNA-reprogrammed T cells are able to recognize HIV-epitopes

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To date it has been demonstrated that HIV-1-specific CD8+ cytotoxic T lymphocytes(CTL) with a broad-specificity, especially against several well known epitopes play an important role in keeping the viral load under control. Unfortunately, not all patients are able to generate such strong immune response against HIV. A potential immunotherapeutic strategy for those patients is the adoptive transfer of T cells, which are reprogrammed by transfection of an HIV-specific T cell receptor (TCR) to eliminate the virus-infected cells. Up to now, such HIV-1-specific reprogrammed CTL were generated through retroviral transfer of TCR-encoding genes, which harbors several challenges (i.e., life-long autoimmunity, activation/inactivation of genes).Consequently, we investigated the transfer of TCR-RNA into T cells by electroporation and chose TCRs that were able to recognize the HLA-A2 restricted HIV pol-peptide IV9 (ILKEPVHGV) and the HIV gag-peptide SL9 (SLYNTVATL). T cells, reprogrammed with these receptors, obtained the ability to release the pro-inflammatory cytokines IL-2, TNF, and IFN-γ simultaneously, showed specific up-regulation of the activation marker CD25, and kept their ability to proliferate after stimulation with peptide-loaded target cells or target cells presenting the natural processed epitopes. In addition, the TCR-reprogrammed CD8+ T cells were capable of specifically lysing target cells pulsed with the corresponding peptides (for at least three days), or target cells presenting endogenously processed epitopes. Moreover, we compared the avidity of our reprogrammed T cells with the parental CTL and could show that the transfected T cells were only one order of magnitude lower in avidity as the parental CTL. Furthermore, the recognition pattern of mutant peptides by the parental clone was preserved in the TCR-RNA-transfected T cells. Taken together, CD8+ T cells, which were transfected with TCR-encoding RNA, may represent a simple and secure alternative to retroviral transduction and has the benefit of a better evaluation of the transferred TCRs.

P088

Soluble CD83 promotes tolerance induction to minor mismatched skin transplants and allogeneic heart transplants in the mouse.

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Soluble CD83 (sCD83) is a novel immuno-modulatory molecule which has been shown to interfere with DC-maturation processes as well as DC-mediated T cell proliferation *in vitro*. Furthermore, using the murine EAE-model it could be shown thatsCD83 is able to inhibit the paralyses associated with this autoimmune disorder. Here we report that sCD83 also interferes with immune processes responsible for the rejection of transplants. Thus, in a minor mismatch skin transplantation model male donor tail skin (Balb/c) was transplanted onto the back of female recipient animals. Recipients were either treated 8 x with sCD83 (100 μg per mouse, day-1–7) day or were left untreated. In 50% of the animals this mono-therapy prevented the transplant rejection completely, whereas all untreated animals rejected their transplant at the latest by day 80. Next, all sCD83-treated animals which did not reject the first transplant were transplanted for a second time and strikingly all the transplants were accepted even dough sCD83 was not applied during this second transplantation, indicating that sCD83 induces regulatory mechanisms, possibly Treg, which prevent transplant rejection. Subsequently, the immuno-modulatory effect of sCD83 was investigated using an allogeneic murine heart transplant model in combination with anti-CD45RB mAb and/or rapamycin. Thus, C3H mouse hearts were heterotopically transplanted into C57BL/6 mice. Without immuno-suppression, heart grafts were rejected in 8.3 0.5 days by acute cellular and humoral rejection. sCD83 mono-therapy(100 μg per mouse, day-1–28) attenuated acute rejection and doubled heart graft survival to 15 10 days. In addition, sCD83 has synergy with subt herapeutic dose of eitheranti-CD45RB mAb (50 mg per mouse, day0-13, i.p.) or Rapamycin (2 mg kg^{-1}, day 0–13, p.o.) to further improve graft survival to 32 3.6 days and 39.3 4.7 days, respectively. Remarkably, sCD83 in combination with both anti-CD45RB mAb and rapamycin effectively prevented acute rejection and achieved graft tolerance with indefinite survival for more than 100 days. Taken together, these data indicate that sCD83 may provide a promising therapeutic approach to induce tolerance in clinical transplantation.

P089 (V23)

MCS-18, a novel natural product isolated from Helleborus purpurascens, inhibits dendritic cell activation and prevents autoimmunity *in vivo*.

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Dendritic cells (DC) as antigen presenting cells play an important role in the initiation of an autoimmune disease like type 1 diabetes. We reported, that MCS-18, a novel natural product isolated from Helleborus purpurascens, is able to inhibit the expression of typical molecules of mature DC such as CD80, CD86, and especially ofCD83 subsequently leading to a clear and dose-dependent inhibition of the DC-mediated T-cell stimulation. Furthermore, MCS-18 also impeded the formation of the typical DC/T-cell clusters. Strikingly, MCS-18 also strongly reduced the paralysis associated with the experimental autoimmune encephalomyelitis (EAE) in aprophylactic as well as in a 'real' therapeutic setting. Even when the EAE was induced for a second time, the MCS-18-treated animals were still protected, suggesting that MCS-18 induces a long-lasting suppressive effect. Here we report for the first time that MCS-18 is also able to increase diabetes free survival using the NOD-mouse model. Interestingly, in the animal group which has been treated withMCS-18 during week 7 and week 15 of age (2 mg per dose per every 2nd day per i.p.) over70% of the animals showed a diabetes free survival at week 30, whereas in sharp contrast in the untreated animals less then 20% were free of diabetes. In addition, insulitis scoring and immune-fluorescence staining revealed that MCS-18 significantly reduced islet T-cell infiltrates. ELISpot analyses showed that MCS-18 treatment resulted in a clearly reduced T-cell proliferation. In conclusion, these studies show that MCS-18 exerts a strong immunosuppressive activity with remarkable potential for the therapy of diseases characterized by a pathologically over-activated immune system. Thus, further development of MCS18-specific therapeutic interventions maybe of benefit for the treatment of autoimmune disease.

P090

S100A7A15 primes and promotes skin inflammation in a psoriasis mouse model

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Psoriasis is genetically linked to the human S100A7/A15 subfamily encoded within the psoriasis susceptibility locus at chromosome 1q21. Inflammation-prone psoriatic skin is characterized by constitutively elevated levels of S100A7/A15 in epidermis. Here we report that bitransgenic mice expressing elevated levels of mS100A7A15 by skin keratinocytes are primed for an exaggerated inflammatory response when challenged by exogenous stimuli (Koebner phenomenon). Inflammation-prone transgenic skin is infiltrated with immune cells and expresses elevated levels of Th1 and Th17 proinflammatory molecules linked to the pathogenesis of psoriasis, which are further amplified upon challenge. Both, inflammation priming and amplification require mS100A7A15 ligand and the receptor of advanced glycated end products (RAGE). mS100A7A15 potentiates inflammation directly as a chemoattractant further enhancing the inflammatory infiltrate in skin from bitransgenic mice. This study models a functional mechanism for a psoriasis candidate gene and emphasizes the link between the epidermal and immune compartments as pathogenetic model for inflammation priming. Thus, targeting S100A7A15-RAGE may be a novel therapeutic approach for treatment of susceptibility and inflammation in psoriasis.

P091

ATP is a tissue site specific activator of naïve regulatory T cells *in vivo*

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The two phases of the murine contact hypersensitivity (CHS) are initiated at different tissue sites. The first antigen contact, the sensitization phase, takes place in the draining lymph nodes, whereas the second application of the antigen induces the immune reaction in the tissue and in the blood. Nevertheless, adoptively transferred naïve CD4+CD25+Foxp3+ regulatory T cells (Treg) are able to suppress both phases of the CHS reaction. Since it has been established that Treg require activation to convey their suppressive capacity, we analyzed the activation status of adoptively transferred Treg after injection *in vivo* during CHS responses. Isolated naïve, fluorescently labelled Treg were injected either 2h before sensitization or 15 min before challenging. CD4+ cells were injected as controls. 24h after sensitization or challenging with hapten, the fluorescently labelled Treg showed increased expression of the activation markers CD69, Foxp3 and CD44 *in vivo*. Because we have recently shown that the suppressive function of Treg is partly mediated by adenosine, generated by degradation of ATP via CD39/CD73, we hypothesized that ATP in addition to serving as substrate for adenosine production, might also act as an activator for Treg. In *in vitro* experiments we demonstrated that ATP indeed induced the activation of naïve Treg as indicated by up-regulation of CD69. Moreover, ATP-activated Treg showed increased suppressive capacity, comparable to results obtained with anti-CD3/CD28 activated Treg. Experiments applying PPADS, a P2 ATP-receptor antagonist, showed that activation as well as the suppressive function of ATP stimulated Treg were abrogated. The *in vivo* relevance of this mechanism was established by findings showing that the application of hapten augmented the ATP concentration in specific tissue. That is, during sensitization ATP was increased in the draining LN, whereas challenge elevated ATP levels selectively in the serum. These data strictly correlate with the observed pattern of Treg activation *in vivo*. Moreover, the suppression of the CHS response by Treg was dependent on functional P2 ATP-receptors, as blockade of these receptors on Treg by PPADS abrogated the suppressive function of the Treg *in vivo*. Thus, these data suggest a critical role of ATP in tissue-specific activation of Treg via P2receptors during allergic and inflammatory reactions.

P092

Endothelial cells augment the suppressive function of CD4+CD25+Foxp3+regulatory T cells: Involvement of PD-1 and IL-10

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Blood endothelial cells (EC) act as gatekeepers to coordinate the extravasation of different T cell subpopulations. To do so, EC express defined panels of adhesion molecules, facilitating interaction with blood circulating T cells. Besides the mere adhesion, this cellular interaction between EC and transmigrating T cells may also provide signals that affect the phenotype and function of the T cells. To test the effects of EC on regulatory T cells (Treg) we set up cocultures of freshly isolated murine Treg and primary EC and assessed the phenotype and function of the Treg. We show that (Treg) up-regulate PD-1 expression, as well interleukin (IL)-10 and TGF-β secretion after contact to EC. These changes in phenotype were accompanied by an increased suppressive capacity of the Treg. Blockade of thePD-1 and/or the IL-10 secretion in *in vitro* suppression assays abrogated the enhanced suppressive capacity, indicating relevance of these molecules for the enhanced suppressive activity of Treg. In aggregate our data show, that endothelial cells increase the immunosuppressive potential of activated Treg by up-regulation ofPD-1 and stimulation of the production of high levels of IL-10 and TGF-β. Therefore one can speculate that Treg during transendothelial transmigration become 'armed' for their suppressive function(s) to be carried out in peripheral tissues sites.

P093 (V16)

Regulatory T cells prevent the priming of CD8+ T cells in contact hypersensitivity reactions by interaction with dendritic cells via gap-junctions *in vivo*

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To analyze the influence of regulatory T cells on CD8+ T cell mediated immune responses *in vivo*, we used the model of hapten-induced contact hypersensitivity (CHS) in mice. CHS is induced by sensitization of individuals against haptens. This sensitization occurs in the draining lymph nodes (dLN) where antigen-specific priming of CD8+ T cells by dendritic cells (DC) takes place. Our initial studies showed that the immune reaction, as measured by the ear swelling response induced by challenging the mice 5d after sensitization, is inhibited by injection of CD62L+, LN-homing CD4+CD25+ regulatory T cells (Treg) before sensitization. In contrast, CD62L- Treg, which fails to migrate to the dLN, also failed to suppress the CHS reaction. CD62L+ Treg migrate to dLN and establish cellular contact to DC, which was demonstrated by fluorescence microscopy of cryosections of dLN stained with fluorescence markers for Treg, DC or CD8+ T cells, respectively. Further detailed analysis of this interaction revealed that fluorescently labelled Treg transfer the cytoplasmic dye to DC *in vivo* and that Connexin 43, a marker for gap junctions, is present at the Treg - DC interfaces. As a consequence of this interaction, DC display reduced expression of CD80, CD86 and are impaired in stimulating hapten-specific effector T cells. Moreover, we detected a significantly increased production of the anti-inflammatory cytokine IL-10 by CD1 1c+ DC after Treg treatment (600 pg mL^{-1}) compared to 'only sensitized' control mice (350 pg mL^{-1}). Blocking of the gap junctional contact between DC and Treg *in vivo* by application of Gap27 peptide, a specific gap-junction inhibitor, abrogated the suppressive function of the Treg during CHS responses. Thus, these data reveal an essential role of the formation of gap junctions between Treg and DCas a novel means of Treg-mediated suppression of immune responses, and we conclude that gap junctional intercellular communication leads to abrogation of the priming, the activation and the proliferation of hapten-specific CD8+ T cells. In aggregate Treg do not only modulate ongoing CD4+ T cell mediated immune reactions at tissue sites but also abrogate the de novo induction of CD8+ T cell driven immune reactions by interfering with T cell stimulatory activity of DC via gap junctional intercellular communication.

P094

The immunohistochemical distribution of the antimicrobial peptides Psoriasin, RNase7, human β-defensin-2 and -3 varies in healthy human skin

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Antimicrobial peptides (AMP) are known to play an important role in the skin's first line of defense against infection. Various AMP have been identified, some constitutively expressed like Psoriasin (S100A7) and RNase 7, others inducible like human β-defensin-2 (hBD-2) and -3. In a previous study we had first evidence that Psoriasin, a potent agent against *E.coli*, displays considerable differences in immunoreactivity between various body sites and age groups. Aim of this study was therefore to analyze systematically the expression pattern of various AMP in healthy human skin derived from different body localizations and from different age cohorts in a representative collective. Formalin fixed and paraffin embedded tissue samples of healthy human skin derived from surgery of benign melanocytic naevi (n = 278) were stained with specific antibodies for Psoriasin, Rnase7, hBD-2 and hBD-3. The test specimens were composed from three age groups (< 20, 20–60, > 60 years), each consisted of five individual samples. A total of 19 different body localizations were analyzed for Psoriasin and RNase7, hBD-2 and -3 were stained for 12 different localizations. Using a standardized scoring protocol, immunoreactivity was evaluated by two independent investigators for every field of view in 400-fold magnification. Variable differences in the staining intensity between the body localizations were shown for Psoriasin and RNase7, whereas hBD-2 and -3 displayed no significant differences. Within the malpighian layers Psoriasin showed the highest staining intensity in the stratum granulosum and stratum spinosum, whereas the highest expression for all other AMP under investigation was found in the stratum corneum. Notable differences in immunoreactivity between the three age groups occurred in specific localizations. This study demonstrates that Psoriasin and RNase7 immunoreactivity displays variable differences in body localizations in contrast to hBD-2 and -3. Considering these findings, the usage of healthy control samples matching in localization and approximate age is highly recommended for comparative immunohistochemical analysis of AMP expression, especially for Psoriasin and RNase7.

P095

Generation of blister-inducing autoantibodies of distinct subclasses and specificity is linked to H2s in an active mouse model of epidermolysis bullosa acquisita

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Epidermolysis bullosa acquisita (EBA) is a severe autoimmune subepidermal blistering disease of skin and mucous membranes, characterized by antibodies to type VII collagen. EBA can be induced in mice by immunization with a recombinant portion of the non-collagenous (NC) 1 domain of murine type VII collagen and reproduces the clinical, histopathological, and immunopathological findings observed in patients (active EBA mouse model). In contrast to other autoimmune disease, e.g. rheumatoid arthritis or lupus erythematosus, little data is available on genetic susceptibility of autoimmune blistering skin diseases. We therefore used the active EBA mouse model to address the hypothesis that disease induction depends on theMHC haplotype. Mice from inbred strains, namely SJL/J (H2s), C57Bl/10.s (H2s), MRL/MpJ (H2k), DBA/1J (H2q), C57Bl/6J (H2b), NZM2410/J (H2z), BXBD/TyJ (H2b), NOD/ShiLtJ (H2g7), and C57Bl/10.q (H2q), were immunized with recombinant murine type VII collagen NC1. Overall, 5 distinct responses were observed: induction of (i) severe and stable disease in SJL/J and female MRL/MpJ mice, (ii) mild and transient disease in C57Bl/10.s mice, (iii) blistering, that could be detected only histopathologically but not clinically in DBA/1J mice, (iv) presence of circulating, non-disease-inducing autoantibodies in most strains, and (v) complete resistance to EBA and lack of autoantibody production in NOD/ShiLtJ and C57Bl/10.q mice. These observations indicate that susceptibility to experimental EBA is strongly associated with H2s. In addition we confirm and extend previous findings, that clinical EBA is associated with the presence of complement-activating antibodies. As binding of autoantigens to the MHC depends on the haplotype, we investigated the specificity of the autoantibody response: This demonstrated that (1) the autoantibody-response was restricted to the peptide sequence of the protein used for immunization, as preadsorption with this recombinant protein completely diminished the reactivity of sera from diseased animals with murine skin by indirect immunofluorescence microscopy; and (2) autoantibody reactivity to 3 specific 20mer peptides of type VII collagen NC1 was observed only in mice with H2s, and was absent in clinically healthy mice. Our findings add to the understanding of the pathogenesis of autoimmune bullous skin disease, and may contribute to the development of more specific therapies for these diseases; e.g. extracorporal immunoapheresis of specific autoantibodies or induction of tolerance.

P096

DEC-205 targeting of dendritic cells: a promising tool for antigen loading?

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To improve the efficiency of dendritic cell (DC)-based vaccination for cancer immunotherapy, we examined antigen-loading by targeting DEC-205 on DC using an antibody-antigen fusion protein, and compared this strategy with direct peptide-loading and electroporation of defined tumor-antigen RNA. As a model antigen, we chose the cancer-testis antigen MAGE-A3. The antibody-antigen construct consisted of a single-chain variable fragment (scFv) directed against DEC-205, genetically linked to a part of MAGE-A3 (aa 243–258), which can be presented on HLA-DP4. The antibody-antigen construct displayed binding to immature (i) DC and mature (m) DC, and specific binding to DEC-205-transfected-CHO cells. The construct alone had no influence on DC phenotype, cytokine secretion, and migratory capacity. In order to analyze whether the DEC-205 targeting of DC led to antigen presentation, we incubated iDC and mDC for 48 h with the fusion protein or a control construct. After 24 h, half of the iDC were matured during the targeting for an additional 24 h (iDC > m). As a control, mDC were electroporated with MAGE-A3-DCLAMP RNA or MAGE-A3 RNA, or were loaded with theMAGE-A3/DP4 peptide or the control peptide NY-ESO-1/DP4. The DC were then used to stimulate MAGE-A3/DP4-specific CD4+ T cells, which were generated by RNA electroporation. Indeed, DEC-205-targeted antigen loading of iDC, mDC, and iDC > m led to antigen presentation. The DEC-205 targeting of iDC > m was significantly superior to direct peptide loading and electroporation. Taken together, we show that DEC-205 targeting is an efficient and promising tool to load DC with tumor antigen. Currently we examine whether DC, generated from malignant melanoma patients, can be loaded with MAGE-A3 by using our construct, in order to investigate whether this strategy is applicable in immunotherapy.

P097

TNF-α-bearing macrophages: A target for TNF-α inhibitors in non-infectious granulomatous diseases?

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TNF-α antagonist have revolutionized the treatment of non-infectious inflammatory skin diseases such as psoriasis, M. Behcet and pyoderma gangrenosum. One serious side effect of this form of treatment is the reactivation of infectious processes such as tuberculosis. In this case, the spread of mycobacteria is apparently due to agranuloma-disrupting effect of anti-TNF-α agents. We therefore reasoned that these agents could be promising tools in the treatment of wide spread, non-infectious granulomatous processes such as disseminated granuloma anulare (GA), sarcoidosis and others. To test this hypothesis, we initiated i.v. treatment with the TNF-α inhibitor Infliximab (5 mg kg^{-1} per day at weeks 0, 2, 6, 14, 22 etc.) in four female patients with disseminated GA. The clinical effects were quite dramatic. GA lesions began to regress already during the first two weeks of treatment and had completely disappeared after 4 administrations of infliximab. By immunohistology, untreated GA lesions displayed increased numbers of CD207+ epidermal Langerhans cells and, most notably, palisading granulomas consisting predominantly of CD11b+/CD14+ macrophages and CD3+ T cells. These latter cell types also exhibited pronounced anti-TNF-α reactivity. For reasons yet unknown, the expression density of MHC class II on LC and macrophages was greatly reduced compared to that of normal skin. After 4 administrations of infliximab, LC and macrophages were greatly decreased and TNF-α expression had disappeared. T cells, by contrast, were still present, albeit at as lightly reduced frequency. We conclude that infliximab is an effective treatment for recalcitrant disseminated GA and that TNF-α-producing macrophages are the main target of its action. It is tempting to speculate that an infliximab-induced macrophage apoptosis might induce immunological events resulting in the down-regulation of the inflammatory process.

P098

Increased skin contact hypersensitivity correlates with altered number and function of regulatory T cells in PPAR-α mice.

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PPAR-α deficiency was previously shown to be pro-inflammatory. However, the underlying mechanism remained unknown. Therefore, the aim of our work was to dissect the cellular basis of the pro-inflammatory effects of PPAR-α deficiency in skin. We found that, after challenge with a contact allergen, cutaneous hypersensitivity reactions were increased in PPAR-α deficient mice when compared to wild type animals. The number of T-lymphocytes was increased in the inflammatory dermal infiltrates, predominantly consisting of CD4+ T-lymphocytes with increased expression of the activation marker CD25 in PPAR-α deficient mice compared to wild type animals. Upon allergen challenge, the percentage of regulatory T cells (Treg) was decreased in the skin draining lymph nodes of PPAR-α deficient mice compared to wild type animals. Production of IL-2 was decreased while production of TGF-β remained unchanged in the skin draining lymph nodes of PPAR-α deficient mice compared to wild types. Injection of IL-2 to mice upon sensitization partially restored the Treg population in PPAR-α deficient mice suggesting that lack of Foxp3 up-regulation in PPAR-α deficient mice upon allergen challenge results from both an intrinsic un ability to up-regulate Foxp3 and alack of IL-2 in the T cell microenvironment. In addition, PPAR-α deficient Treg exhibited dysfunction since their suppressive capacity was impaired *in vitro* and *in vivo* after adoptive transfer when compared to wild types. In conclusion, PPAR-α deficiency aggravates skin contact hypersensitivity by triggering Treg.

P099

Induction of antimicrobial peptide expression by adipokines in human keratinocytes

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Psoriasis is a chronic inflammatory disorder which is characterized by an activation of the innate immune system. A hallmark of psoriasis is the up-regulation of antimicrobial peptides (AMP) in the lesional psoriatic epidermis. Epidemiological studies have clearly demonstrated that in western countries the majority of psoriasis patients are obese. Adipocytes of the central body fat produce a number of mediators referred to as adipokines. The spectrum of adipokine secretion is different in obese as compared to normal weight patients. Recent results showed increased levels of leptin and reduced levels of adiponectin in the peripheral blood of psoriasis patients. The aim of the present study was therefore to explore if adipokines may influence the expression of AMP and thus contribute to the abundance of AMP in psoriasis. Primary human keratinocytes derived from foreskin were incubated with four different human adipokines (adiponectin, leptin, resistin or visfatin) and a medium control for different time points. After mRNA isolation and cDNA-synthesis expression of AMP (human β defensin (hBD)-2, -3, psoriasin and RNase 7) was analyzed by real-time PCR. The results showed a time-dependent up-regulation of hBD3 and RNase 7 by adiponectin and leptin in comparison to the medium control whereas resistin and visfatin did not influence mRNA expression. These data demonstrate that adipokines are able to induce the gene expression of AMP, in particular hBD-3 and RNase 7, in human keratinocytes. Further studies have to evaluate the role of adipokines in cutaneous innate immunity.

P100

Development of atopic dermatitis-like inflammation is critically dependent on Langerhans cells in mice.

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Although skin dendritic cells (DC) are suspected to be involved in atopic dermatitis (AD), direct evidence of a pathogenetic role for skin DC in thymic stromal lymphopoietin (TSLP)-induced skin inflammation has not yet been demonstrated. High TSLP expression was found in epidermis from AD patients. Similarly, genetic or vitamin D3-induced over expression of TSLP by keratinocytes resulted in AD-like inflammatory phenotype in mice. We have shown that epidermal Langerhans cells (LC)-depleted mice treated with a vitamin D3 analogue (MC903) do neither develop AD-like inflammation nor increased serum IgE as compared to vehicle-treated mice. Furthermore, expression of maturation markers by LC was increased whereas maturation of dermal DC was not altered. Only LC was responsible for the polarization of naïve CD4+ T cells to a Th2 phenotype i. e., decrease in IFN-γ and increase in IL-13 production by CD4+ T cells. This effect of LC on T-lymphocytes did not require OX40-L/CD134 and was mediated by a concomitant down-regulation of IL-12 and CD70. While it was previously stated that TSLP up-regulates the production of TARC/CCL17 and MDC/CCL22 by human LC *in vitro*, our work shows that production of these Th2- cell attracting chemokines was increased in keratinocytes in response to TSLP over expression. In conclusion, we have shown for the first time the critical implication of LC in the development of AD, their ability to induce the Th2 response by the CD4+ T cells and the essential role of TSLP in triggering AD via LC. Based on these findings it is tempting to speculate that inhibiting LC function at early stages of AD development could be a particularly effective strategy.

P101

Depletion of DEC205+ dendritic cells by a specific single-chain fragment variable (ScFv) toxin

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Dendritic cells (DC) are divided into two major subsets that differ in the expression of the surface molecules CD8, DCIR and DEC205. CD8+ DCIR+ DC induces Th2 responses, whereas CD8– DEC205+ DC is known to induce IFNγ-producing Th1 T cells. In addition, only the latter subpopulation is able to induce FoxP3+ Treg in the absence of exogenous IL-2 or TGF-β. To minimize tolerogenic effects of DEC-205+DC during cancer therapy, we devised a novel method to eliminate this tolerogenic DC subset *in vivo*. Therefore, we generated a single-chain fragment variable (ScFv) specific for murine DEC205, based on a monoclonal anti-DEC205 antibody. This ScFv was fused to Pseudomonas aeruginosa Exotoxin A, an inhibitor of ribosome. So the uptake of this toxin finally induces cell death. The DNA sequences of both, the ScFv and ETA were cloned into an expression vector fused to a 6×His tag and a c-myc tag. We cloned two variants of this construct, fusing ETA to the 5′-end or at the 3′-end of the ScFv. The production of the recombinant proteins was done with the *E. coli* strain TG1 by periplasmatic expression, induced by IPTG. Purification of the proteins was achieved by affinity chromatography, followed by dialysis. To address whether the ScFv bind to DC, we incubated bone marrow derived DC (BMDC) with ScFv-ETA and subsequently stained with a c-myc specific antibody. Immunohistochemistry analysis indicated effective binding to the DEC205 receptor of both of the constructs. In order to reveal functionality of the ScFv-ETA, BMDC were incubated with graded doses of ScFv-ETA and subsequently induction of cell death was investigated by PI staining. Hereby we could clearly show that the ScFv-ETA is able to ablate DC. Specificity of the toxic effect was validated by incubation ofDEC205- fibroblasts with ETA-ScFv that did not lead to apoptosis.
Thus, ScFv-ETA is an efficient tool to eliminate DEC205+ DC and further experiments will reveal the role of DEC205+ DC in tolerance and immunity.

P102 (V29)

FcgammaRIV promotes, while FcgammaRIIB protects from autoantibody-induced tissue damage in autoimmunity to type VII collagen

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Epidermolysis bullosa acquisita (EBA) is an autoimmune bullous disease mediated by autoantibodies against type VII collagen, the major component of anchoring fibrils of the dermal-epidermal junction. When passively transferred into mice, rabbit IgG against type VII collagen induces Fc-dependent activation of complement, the recruitment of leucocytes into the skin, and sub-epidermal blistering. Since co-expression of activating (FcgammaRI, FcgammaRIII, and FcgammaRIV) and inhibitory (FcgammaRIIB) Fc receptors (FcRs) is believed to represent an immunoregulatory checkpoint by establishing a threshold for immune cell activation, we determined the role of different FcgammaRs on tissue damage in experimental EBA. Mice lacking the common gamma-chain of activating FcRs (FcRgamma) were completely resistant to experimental EBA induction. Regarding the contribution of the3 different activating FcgammaRs, by use of knock out mice or function blocking antibodies, we showed a significant contribution of FcgammaRIV. More specifically, only one of six FcgammaRIV-deficient mice developed mild EBA, whereas all control mice presented with severe skin lesions. In contrast, FcgammaRI or FcgammaRIII deficiency had no or little effect on disease expression, respectively. In addition, FcgammaRIIB deficiency was associated with significantly enhanced disease severity. Our observations suggest targeting of FcgammaRIV and FcgammaRIIB as potential therapeutic options in EBA.

P103

Upregulation of TLR2 expression in keratinocytes in barrier disruption is caused by cytokines

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The innate immune system supports the physical skin barrier in antimicrobial defense. Human keratinocytes constitutively express various members of the toll-like receptor (TLR) family, important for innate immunity including TLR2. TLR2 recognizes pathogen associated molecular pattern of gram-positive bacteria such as lipoteichoic acid. We asked whether TLR2 expression is increased after acute skin barrier disruption in mouse skin *in vivo* and by cytokines in human keratinocyte culture *in vitro*. The expression of human and murine TLR2 was assessed by real time PCR analysis and subsequent gel electrophoresis for verification as well as by immunohistochemistry. Acute barrier injury by scraping of mouse skin enhanced expression levels of TLR2 at 6 h after treatment. Chronic skin barrier disruption by an essential fatty acid diet (EFAD) in mice also resulted in an enhanced TLR2 protein expression when compared with untreated skin. Interleukin 1 beta and interfere on gamma induced TLR2 expression in keratinocyte cell culture. As interferon gamma is a typical Th1 cytokine upregulated in acute skin barrier disruption it may be responsible for the induction of TRL2 expression seen in acute and chronic barrier disruption, which resembles acute and chronic eczema.

P104

A common haplotype of the IL-31 gene is related with higher serum IL-31 levels and increased pruritus in adult atopic dermatitis patients

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IL-31 is a cytokine that is over expressed not only in transgenic mice, in which it induces severe itch, but also in human pruritic skin diseases like prurigo nodularis or atopic dermatitis. Recently, a strong association of a common IL-31 haplotype (haplotype A) with the intrinsic type of atopic dermatitis has been reported. We aimed to investigate a possible relation between variants in the IL-31 gene, serum IL-31levels, and different disease features in atopic dermatitis patients including disease severity, pruritus, and type I sensitizations. Therefore, the previously described polymorphisms in the IL-31 gene were analyzed by pyrosequencing in a cohort of 73 adult atopic dermatitis patients. Patients with the haplotype A were compared with non-A carriers regarding serum IL-31 levels by ELISA, average pruritus as analyzed using the visual analogue scales (VAS), average SCORAD over 2 years, and type I sensitizations detecting total IgE and specific IgE to house dust mite, birch pollen, and timothy grass pollen. 19 of 73 patients were positive for haplotype A (26%).These patients showed significantly higher serum IL-31 levels and average itch values measured by VAS over a period of 2 years. In contrast, neither relation was seen between haplotype A and disease severity as measured by mean SCORAD over a 2 year period, nor total and specific serum IgE levels.

The relation between haplotype A and higher serum IL-31 levels may be due to activation in the promoter region. Consistent with the known involvement of IL-31 in itching skin diseases, these patients also suffered from more itch. We conclude thatIL-31 variants play an important role in the pathogenesis and severity of itch, whereas they are no major risk factors for severe atopic dermatitis associated with high immediate type sensitization levels in adulthood.

P105

Molecular mimicry in pemphigus vulgaris - T cell recognition of desmoglein 3epitopes and unrelated homologous peptides

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Pemphigus vulgaris (PV) is caused by IgG against the epidermal adhesion molecule, desmoglein 3 (Dsg3). We have shown that PV patients show autoreactive T cells against Dsg3, which are tightly restricted by PV-associated HLA class II alleles. Moreover, T cell recognition of a limited set of Dsg3 epitopes is confined to the presence of distinct binding motifs of these peptides to these afore mentioned HLA class II alleles. Priming of autoagressive effector T cells in PV may involve molecular mimicry between peptide fragments of unrelated proteins which may later on lead to a pathogenic T cell response against epitopes of Dsg3. Upon searching databases of the human genome and other species, a set of peptides homologous to immunodominant Dsg3 peptides was identified based on HLA-DRβ1*0402-specificbinding motifs. All the identified Dsg3 peptides contained conserved amino acids at relative positions 1, 4 (positive charge), and 6 (7) that presumably represent anchormotifs for DRB1*0402 and DQB1*0503. Furthermore, T cell receptor contact sites were contained at positions -1, position 0, and at positions 2 or 3, respectively. EightDsg3-specific T cell clones specific for the immunodominant Dsg3 epitopes, DSG3 (96–112), DSG3 (250–266), or DSG3 (376–392), were co-cultured with 49 homologous peptides to identify their stimulatory function as mimicry peptides. Although the majority of the homologous peptides did not stimulate the Dsg3-specificT cells, three autoreactive T cell clones responsive to DSG3 (96–112) were also stimulated by a homologous peptide derived from human DSG1, i.e. DSG1 (96–112). This finding provides an explanation why the majority of patients with PV also show IgG against Dsg1 during the chronic disease course. Three T cell clones specific for the Dsg3 epitope, DSG3 (376–392), were also stimulated by homologous peptides from *Clostridium tetani* and HIV-1 virus. These observations provide evidence that molecular mimicry occurs at the T cell level potentially leading to clinical flares (induced by infectious peptides) or epitope spreading (shifted T cell recognition fromDsg3 to Dsg1 epitopes) leading to a more extensive clinical phenotype.

P106

Dissecting early and late phase mast cell activation pathways: Serum- and glucocorticoid-inducible kinase 1 is critical for early but dispensable for delayed type mast cell responses

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Mast cell activation determines IgE mediated immediate type allergic reactions and also some prototypic T cell mediated delayed type hypersensitivity responses. It was still undefined whether early and delayed type activations of mast cells are consecutive events or independently regulated. Among others, allergen-IgE-mediated cross linking of FcεRI receptors activates the phosphoinositol-3(PI3)-kinase pathway. It is known that the serum- and glucocorticoid-inducible kinase1 (SGK1) is activated by this PI3-kinase pathway. We aimed to investigate the role ofSGK1 for early and delayed type mast cell responses. SGK1 knockout (sgk1–/–) mice and wild-type littermates were passively sensitized with Dinitrophenol (DNP)-specific IgE and 24 h later challenged with DNP to elicit anaphylaxis. The decrease of core body temperature as read-out for the strength of the anaphylactic reaction was detected by measuring rectal temperature every 7 minutes. Anaphylaxis with a fast decline in body temperature (1.74°C ± 0.41°C) was detected in sgk1+/+ mice only, while sgk1–/– mice showed no anaphylactic reaction. In line with this, degranulation as detected by release of β-hexosaminidase was significantly reduced in bone marrow derived mast cells (BMMC) from sgk1–/– mice. However, BMMC of sgk1–/– and sgk1+/+ mice showed unequivocal phenotypes after 4 weeks of culture with IL-3and SCF regarding β-hexosaminidase content and expression of CD117, CD34 and FcεRI. With patch clamping we identified reduced early BMMC cell membrane hyperpolarisation following allergen specific activation exclusively in sgk1–/– BMMC secondary due to impaired Ca2+ influx in these cells. Next, mast cell dependent contact hypersensitivity (CHS) response to TNCB was investigated. Ear thickness was determined before and 4, 8, 12, and 24 h after TNCB application in sensitized mice. Strikingly, ear swelling was significantly impaired in sgk1–/– compared to sgk1+/+ mice only during the early response (4 and 8 h) where as no difference between CHS responses was detected at 24 h. This indicates that different mast cell activation pathways are critical for early and delayed responses even following the same activation stimulus. In agreement with these data we could show that, following different stimuli, SGK1 is crucial for early cytokine release (6 h), but not for delayed type cytokine secretion (24 h) by BMMC. In conclusion, our data demonstrate for the first time that consecutive early and delayed type mast cell activation pathways can be regulated independently even following the same stimulus. Based on this specificity, targeting SGK1 may be highly promising to treat immediate type allergic responses while sparing critical delayed type mast cell responses.

P107

In vivo conversion of CD4+FoxP3– to CD4+FoxP3+ T cells

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FoxP3+ naturally occurring regulatory T cells (nTreg) are potent suppressors of autoreactive CD4+ T cells that escape negative selection in the thymus. The fork head transcription factor FoxP3 is indispensable for the differentiation, maintenance, and function of nTreg. TCR stimulation of conventional CD4+FoxP3- T cells *in vitro* in the presence of TGFβ and IL-2, results in the induction of FoxP3 expression. These TGFβ -induced FoxP3+ T cells (iTreg) are identical to nTreg in that they are anergic and suppressive *in vitro* and both, polyclonal and antigen-specific iTreg, have demonstrated anti-inflammatory potential in animal models of organ-specific autoimmune disease.

To address the question if the conversion to CD4+FoxP3+ T cells also takes place *in vivo*, we transferred sorted CD4+GFP- cells from the Foxp3-GFP 'knock-in' mice intoRAG1–/– mice and found that a substantial number of cells (1.15% ± 0.18% FoxP3+within the CD4+ T cells) converted to GFP+ cells within 4 weeks. In contrast no conversion was detectable after transfer of CD4+GFP- cells into WT recipients. The percentage of converted GFP+ T cells was higher in the peripheral lymph nodes than in the spleen or the skin, with the highest percentage in the mesenteric lymph node (2.1% ± 0.32% FoxP3+ within the CD4+ T cells). The *in vivo* converted GFP+ T cells were suppressive *in vitro* when sorted directly *ex vivo* 4 weeks after original transfer into RAG1–/– recipients. In summary, these data suggest that certain conditions (e.g. lymphopenia) favor the conversion of FoxP3– cells to FoxP3+ Tregs and that these peripherally converted CD4+FoxP3+ T cells may contribute to the maintenance of tolerance *in vivo*.

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P108

Autoantibodies in Scurfy mice and IPEX patients recognize keratin14

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Scurfy mice have a deletion in the fork head domain of Foxp3 and fail to develop thymic-derived Foxp3+ regulatory T cells. Early in life, Scurfy mice develop a fatal lympho-proliferative syndrome with multi-organ inflammation and the generation of autoantibodies. In humans, loss-of-function mutations in the foxP3 gene lead to asimilar disease, termed the IPEX syndrome (Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked). The skin is affected in all Scurfy mice and most IPEX-patients and histology shows a lymphocytic infiltration in the dermis and epidermis.

The aim of this study was to identify the target antigen(s) in the skin recognized by autoantibodies in serum of Scurfy mice. On Western blot analysis, Scurfy serum, but not WT littermate serum, recognized several bands in total skin lysate from a RAG–/– mouse. Immunohistochemistry on frozen sections of RAG–/– skin revealed that the antibodies in the serum recognized a target in the epidermis. These data were confirmed by Western blot using a murine keratinocyte lysate. Further analysis using 2D-Western blot technology followed by Mass spectrometry identified several keratins as targets recognized by the autoantibodies in Scurfy serum. One of the targets was keratin-14. To confirm this observation and to identify the epitopes recognized by the autoantibodies on keratin-14, we utilized a bacterial expression system to express three polypeptides encompassing the N-terminal, middle, and C-terminal portions of the keratin-14 protein. By Western blot, we found that Scurfy serum predominantly recognized the C-terminal fragment. Our results indicate that keratins, especially keratin-14, are antigenic targets for autoantibodies developing in autoimmune skin disease in the scurfy mouse.

Next we tested the 3 protein fragments covering the whole keratin 14 protein by Western blot analysis with the IPEX patient serum. As in Scurfy mice, the C-terminal fragment of keratin-14 was predominantly recognized by the patient serum.

In summary we show that autoantibodies from Scurfy mice and IPEX patients recognize different keratins including keratin 14 and conclude that keratin 14 is an antigenic target in autoimmune skin disease.

P109 (V09)

The chemokine I-TAC (CXCL11) is an early component of Th2 type immunity

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Differentiation of T-helper (Th) cells into different subsets has a major impact on the course of diverse inflammatory diseases. Experimental leishmaniasis is an excellent model system to investigate the mechanisms underlying Th-cell differentiation *in vivo*. A Th1 response is critical for protective immunity in genetically resistant C57Bl/6mice. Susceptible BALB/c mice on the other hand develop a Th2 response and succumb to progressive disease. The decisive events for the development of a Th1or Th2 response take place during the first two days after infection. There is growing evidence that the microenvironment of the infected tissue delivers the initial triggers that affect Th-cell differentiation.

To identify early components of Th2-immunity we analyzed differential gene expression 16 h after infection with Leishmania (L.) major in the skin of susceptible BALB/c compared to resistant C57BL/6 mice. We used microarray technology (Affymetrix) and bioinformatical analysis (GenMAPP, Gene data Expressionist) to detect L. major induced gene expression patterns in the skin of Balb/c and C57Bl/6 mice 16 h after infection.

In susceptible, but not in resistant mice, we found a marked up-regulation of the chemokine I-TAC (CXCL11) in infected footpads during the first two days of infection. Using laser-micro dissection, *in-situ*-hybridization and immunohistochemistry we revealed that keratinocytes were the major source for local I-TAC production. Treatment of resistant mice with I-TAC at the first day of infection resulted in subsequently decreased levels of the important Th1-instructing cytokine IL-12 in draining lymph nodes and a Th2 shift. I-TAC also inhibited L. major induced IL-12 production by dendritic cells *in vitro*. *In vivo* the I-TAC-induced Th2 switch was not limited to L. major infection but also occurred in subcutaneous vaccination experiments. Most important, a single local injection of I-TAC in resistant mice at the first day of infection resulted in a Th2-driven, dramatic deterioration of disease for more than 6 weeks and in dramatically (>1000-fold) enhanced parasite levels.

This is the first identification of an early signal produced by keratinocytes which instructs systemic Th2-differentiation in experimental leishmaniasis. The Th2 instructing capacity of I-TAC is integrated by DCs via suppression of IL-12 which in turn promotes Th2-ploarization.

P110

Keratinocytes determine Th1/Th2 dichotomy during early experimental leishmaniasis

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Experimental leishmaniasis is an excellent model system for analyzing Th1/Th2 differentiation. Resistance to Leishmania (L.) major depends on the development of a L. major specific Th1 response, while Th2 differentiation results in susceptibility. There is growing evidence that the microenvironment of the early affected tissue delivers the initial triggers for Th-cell differentiation. To analyze this we studied differential gene expression in infected skin of resistant and susceptible mice 16 h after parasite inoculation.

Employing microarray technology, bioinformatics, laser-micro dissection and *in-situ*-hybridization we found that the epidermis was the major source of immunomodulatory mediators. This epidermal gene induction was significantly stronger in resistant mice and encompasses several genes known to affect Th1/2 differentiation such as IL-12, IL-1β, osteopontin, IL4 and IL-6. Their expression was temporally restricted to the crucial time of Th1/2 differentiation. In experimental leishmaniasis IL-12, IL-1β and also IL-4 (later on the classical Th2 cytokine) have been demonstrated to induce a Th1 response when present in an initial and temporally restricted phase of infection. Moreover, using bone-marrow chimeric C57BL/6 mice we now demonstrate that an exclusive loss of IL-6 in non-hematopoietic cells (including keratinocytes) results in a reduced Th1 response and increased susceptibility during L. major infection.

Thus, our data indicate for the first time that epidermal cytokine expression is a decisive factor in the generation of protective Th1 immunity and contributes to the outcome of infection with this important human pathogen.

P111

Myeloid cells, and not T cells, are the main source of TNF-α in plaque-type psoriasis

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The spectrum of tumor necrosis factor (TNF)-α-producing cells is not clearly defined in psoriasis. The elucidation of this question should allow us to better understand the mode of action, efficacy and, perhaps, also the risks of an anti-psoriatic therapy with TNF-α-antagonists.

Using conventional immunofluorescence methods, we were not able to detect TNF-α in sections of lesional psoriatic skin, but by the application of a tyramide amplification system we obtained reproducible and firm staining.

TNF-α was exclusively found on dermal leukocytes coexpressing CD11c and HLA-DR and, to a lesser extent, CD163. This marker profile is consistent with that of mDCs and macrophages.

We did not find TNF-α colocalization on mast cells (CD117+), T cells (CD3+),neutrophils (CD15+HLA-DR-), endothelial cells (vWF+), pDCs (BDCA-2+) or Langerhans cells (langerin+). Consistently, we found corresponding populations of TNF-α-producing mDCs and monocytes in unstimulated PBMCs of psoriatic patients. More importantly, their number closely correlated with disease activity. In healthy persons, anti-TNF-α-stainings of skin and blood yielded essentially negative results.

In vitro, we confirmed that TNF-α-antagonists are able to induce apoptosis in, as well as complement killing and antibody-dependent cellular cytotoxicity of TNF-α producing cell lines. *In vivo*, in fliximab therapy reduced the number of TNF-α-producing cells in the peripheral blood of psoriatic patients 24 h after administration.

Our data strongly suggest that myeloid cells (dendritic cells, monocytes/macrophages) are the main source of TNF-α in stable plaque-type psoriasis. This highlights the importance of these cells in disease pathogenesis.

P112 (V04)

Transfer of mRNA encoding chimeric antigen receptors specific for MCSP intoCD4+ and CD8+ T cells

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Adoptive transfer of bulk T cells with a tumor antigen-specific T-cell receptor (TCR) is an innovative and promising approach to treat malignancies. Chimeric antigen receptors (CAR), which consist of a ScFv directed against a cancer surface antigen and the signalling domain of CD3-zeta and usually also of CD28 molecules, are an attractive alternative for normal TCR since MHC-restricted antigen presentation is not required. To avoid persistent auto-aggression, a reported life threatening risk of engineered T cells with constitutive CAR expression, we explored mRNA electroporation for transient receptor expression in human T cells.

CAR, consisting of different antigen-binding and signalling domains, specific for melanoma-associated chondroitin sulfate proteoglycan (MCSP), which is expressed on melanomas, basal cell carcinomas, and some forms of childhood leukemia, were efficiently transfected into CD4+ and CD8+ T cells. Expression kinetics of the CAR was studied, and at day 9 after electroporation CAR-expression had disappeared. Upon specific stimulation with MCSP+ tumor cells, transfected CD4+ and CD8+ T cells secreted the cytokines IL-2, TNF-alpha, and IFN-gamma. Moreover, the reprogrammed T cells were capable of killing target cells in an antigen-specific manner. The comparison of different CAR showed that using a binding domain with a higher affinity and stability improved the recognition of tumor cells. Furthermore, the incorporation of a CD28 signaling domain sometimes improved the CAR surface expression, and always improved the lytic capacity and cytokine secretion of the T cells.

In aggregate, this study shows for the first time a direct comparison of CAR with different ScFv specific for the same antigen. Furthermore, RNA electroporation provides us with a technique to rapidly compare and choose CAR best suited for the immunotherapy of cancer.

P113 (V05)

A xenograft model of human skin to study the role of antigen-presenting cells in inflammatory skin disease

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Dendritic cells (DCs) of the skin, e.g. Langerhans cells (LCs) of the epidermis, are potent antigen-presenting cells (APCs) and therefore play a critical role in T-cell mediated inflammatory skin disease and allogeneic skin reactions like graft-versus-host disease (GVHD). Manipulation or depletion of antigen-presenting cells may be crucial to prevent skin inflammation. However, many potential APC-modifying agents like monoclonal antibodies are species-specific and preclinical models are lacking.

We therefore generated a model system using human skin xenografts transplanted to NOD/LtSz-scid IL2Rg-null (NSG) mice lacking T cells, B cells and NK cells. Excess skin material obtained from surgical interventions with the patients' informed consent was depleted of the subcutaneous tissue. Slices of these grafts were transplanted between the scapulae of NSG mice. First, we analyzed the impact of xeno-transplantation on the distribution of LCs as well as dermal HLA-DR positive cells in the engrafted human skin. During wound healing, LCs vanished from the epidermis after the second week and were undetectable until 6–8 weeks after transplantation. In contrast, HLA-DR positive dermal cells were present at any time after transplantation. The re-establishment of human LCs in the xeno transplants supports the hypothesis of a local LC-reconstituting population that is independent of precursors from the peripheral blood.

To induce T-cell mediated inflammation, we used DCs generated from peripheral blood of the skin-donors as stimulators in mixed lymphocyte DC cultures (MLDC) withCD8 T cells of a HLA-class I mismatched healthy donor. Allo-reactive T cells were injected into the tail veins of mice in escalating doses. In mice transplanted with healthy skin, an erythematous reaction in the xenograft was detected approximately1 week after the first dose of MLDC T cells. We further detected histological signs of acute skin GVHD (dermatitis, fissuring, single cell apoptosis) as well as infiltrating T cells and a loss of LCs. This observation is consistent with studies showing that infiltrating allo-reactive donor T cells deplete persisting host APCs. In contrast, using skin from an area of previous gamma-irradiation lacking LCs, we were not able to induce skin rash or histological signs of GHVD by injection of allo-reactive T cells.

In summary, we introduce a model-system for inflammatory skin disease using human xenotransplantats on NSG mice. We provide data supporting the hypothesis of a local LC-repopulating precursor population in human skin. In addition, we establish a model of allo-reactivity in xenografts to analyze the role of APCs in T-cell mediated inflammatory skin disease.

P114

Improvement of intradermal immunization against melanoma

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Introduction: Gycolipid antigens are currently tested as adjuvant for immunotherapy as they are able to enhance T cell responses after being presented by dendritic cells to natural killer T cells. We were interested in examining the potential of glycolipid antigen as adjuvant for skin immunization against melanoma. In addition, we investigated if targeting antigen to skin dendritic cells with an antibody would improve T cell responses.

Methods: We measured T cell responses after intradermal immunization of mice with the synthetic glycolipid α-Galactosylceramide (α-GalCer) plus the model antigen ovalbumin protein (OVA) or OVA conjugated to an antibody against the surface molecule DEC-205/CD205. OVA was used together with α-GalCer for immunization against murine OVA-expressing B16-melanoma (B16.OVA). The involvement of skin dendritic cells in this process was tested by removal of the immunization site and with transgenic mice.

Results: Intradermal immunization with α-GalCer plus OVA strongly enhanced endogenous CD8+ T cell responses. As a consequence the growth of transplanted B16.OVA melanoma cells was inhibited in mice. Skin dendritic cells were not involved since depletion of skin dendritic cells did not alter cytotoxic immune responses after intradermal immunization with α-GalCer and OVA. Targeting the same antigen to skin dendritic cells with an antibody against DEC-205 allowed using 1000-times less antigen to obtain similar inhibition of tumor growth.

Conclusion: Thus, the glycolipid α-GalCer is a useful adjuvant for intradermal immunization strategies and in combination with targeting of antigen to skin dendritic cells inhibits tumor growth even with small amounts of antigen.

P115

Subcutaneous infection with *S. aureus* in different mouse strains reveals that resistance is associated with faster influx of neutrophils and Th2 response

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S. aureus frequently colonizes normal skin, nasal mucosa, and wounds that do not reveal signs of overt infection, but still is the leading cause of bacterial skin infection. When infection develops, it can remain locally controlled, or spread peripherally into the dermis (soft tissue infection), and even reach the bloodstream (bacteraemia, sepsis). These different courses depend not only on virulence factors of *S. aureus* but also on host-specific immune response of the epithelial barrier as well as on leukocytes.

To elaborate host-specific differences in the course of infection we inoculated different inbred strains of mice subcutaneously with *S. aureus* strain SH1000.

We found that C57Bl/6 mice were more susceptible than BALB/c and DBA/2 mice. Higher susceptibility was reflected by higher footpad swelling and transient systemic dissemination. Analysis of serum cytokine level revealed differences in production of proinflammatory cytokines, such as IL-1β, IL-6 and MRP8/14, among different inbred strains of mice, indicating a systemic response. Resistance in BALB/c and DBA/2mice correlated with higher expression of chemokine KC and higher influx of neutrophils to the site of infection.

Since *S. aureus* infection persisted more than 2 weeks in our model we analysed T cell response to *S. aureus* antigen. Strikingly we found that infection with *S. aureus* induces an antigen-specific T cell response. Resistant strains developed a Th2 cell response, while C57Bl/6 mice showed a Th1 cell response. Thus Th2 response coincides with significantly reduced footpad swelling in BALB/c and DBA/2 mice upon subcutaneous infection with *S. aureus*. This is the first time to describe that Th2 response can be beneficial for the outcome of *S. aureus* infection.

P116

Mapping of B cell epitopes on desmoglein 3 in pemphigus vulgaris patients by the use of overlapping peptides

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Pemphigus vulgaris (PV) is a severe autoimmune blistering disease associated with autoantibodies to desmoglein 3 (Dsg 3), a transmembrane glycoprotein of the cadherin family. By the use of recombinant fragments of Dsg 3 and a competition ELISA, previous analyses have identified major B cell epitopes on the extra cellular domain (EC) 1 of Dsg 3 in PV patients. Here, we generated 254 overlapping synthetic peptides of 14 amino acids length covering the entire Dsg 3 ectodomain. Each peptide was N-terminally biotinylated and subsequently bound to streptavidine-coupled 96-well plates. Sera of PV patients ($n = 10$) and healthy volunteers ($n = 10$) were then tested for reactivity with the peptides by ELISA. Heuristic data analysis identified six major antigenic sites harbored within the EC1 and EC3 domain of Dsg 3. In order to validate these results, ELISA was performed with larger peptides of 25–30 amino acids in length that covered the initially recognized smaller immunodominant peptides. Sera of 17 additional PV patients and 20 healthy blood donors were tested and a peptide within EC3 was confirmed with the strongest reactivity. To explore the pathogenic relevance of this finding, the identified peptide will be used to purify and preadsorb anti-Dsg 3 autoantibodies for further analysis in the keratinocyte dissociation assay and neonatal mouse model of PV. Our results may be helpful to design more specific therapeutic approaches for PV.

P117

Modulation of neutrophil effector functions by methylprednisolone

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Bullous pemphigoid (BP) is an autoimmune blistering skin disease caused by autoantibodies against type XVII collagen. After binding to type XVII collagen autoantibodies induce neutrophil recruitment and activation, ultimately leading to autoantibody-induced tissue damage. Previous studies revealed that the dermal-epidermal separation is mediated by the production of reactive oxygen species (oxidative burst) and the release of proteases by neutrophil granulocytes. Glucocorticoids such as methylprednisolone (MP) are commonly used in the treatment of BP. In the present study we investigated whether a direct inhibitory effect on granuloyte functions causes the therapeutic effect of MP in BP. In an *ex vivo* cryosection assay MP significantly inhibited antibody-mediated split formation at the dermal-epidermal junction. In addition, MP inhibited the oxidative burst and IL-8release of human neutrophils after exposure to immune complexes *in vitro*. These findings demonstrate that MP directly affects neutrophil functions. Investigating signalling pathways revealed that MP inhibits Erk1/2 MAP kinase and Akt signaling in neutrophils. Pharmacological inhibition of the Erk1/2-MAPK or Akt pathways inhibited immune complex induced oxidative burst of neutrophils *in vitro*. Importantly, inhibition of the Erk1/2-MAPK and Akt pathways resulted in reduced dermal-epidermal split formation *ex vivo*. These data suggest that Erk1/2-MAPK and Akt pathways are potential targets for novel therapeutic strategies for the treatment of BP.

P118

CXCR3-deficiency leads to reduced IgG1 autoantibodies but does neither improve kidney pathology nor infiltration with T cells and plasma cells in NZB/W mice

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NZB/W mice develop a disease very similar to human systemic lupus erythematosus (SLE), characterized by the production of autoantibodies, hyper-γ globulinaemia and inflammation of multiple tissues including skin and kidneys. T cell-dependent production of autoantibodies is responsible for systemic autoantibody-immunocomplex deposits inducing an inflammatory cascade. Later, local inflammation and tissue destruction is further increased by accumulating macrophages, B cells, T cells and plasma cells. A fraction of activated T cells and plasma cells express the chemokine receptor CXCR3 and thus can migrate against the corresponding ligands, the chemokines CXCL9, CXCL10 and CXCL11, which are expressed in sites of inflammation. This receptor/ligand axis is believed to be important for the accumulation of these cells within sites of inflammation. Therefore, blockade of CXCR3 and its ligands is considered a therapeutical option for treatment of inflammatory autoimmune diseases. Accordingly, CXCR3 expressing T cells and plasma cells are enriched within the inflammatory infiltrates in NZB/W kidneys compared to spleen. However, CXCR3 deficient NZB/W mice developed proteinuria -a measurement for kidney destruction - and had inflammatory T cell and plasma cell infiltrates similar to that of NZB/W wild type mice. Nevertheless, CXCR3 deficiency lead to a reduced production of IgG1 anti-DNA antibodies that are believed to be non-pathogenic, while the production of pathogenic IgG2a and IgG2b anti-DNA antibodies were comparable to wild-type animals. These data suggest that blockade of CXCR3 is not an option for treating autoimmune disease mediated by IgG2a andIgG2b antibodies. However, CXCR3 seems to be directly or indirectly involved in the regulation of antibody isotype switching to IgG1.

P119

Antigen targeting to human C-type Lectin DCIR elicits CD4+ T cell responses *in vitro*

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Dendritic cells (DCs) play a very important role as antigen presenting cells in the immune system. In humans and mice several DC subsets have been identified that differ in their antigen presentation capacity and in the initiation of T cell responses. In peripheral tissues immature DCs continually recirculate as sentinels and search for invading pathogens. An encounter of antigen in the presence of microbial products induces the maturation of DCs. Costimulatory molecules such as CD80, or CD86become upregulated while the endocytotic capacity of mature DCs is drastically diminished. In addition, mature DCs release proinflammatory cytokines to attract other inflammatory cells, and antigens are presented to T cells as peptide MHC complexes. Furthermore, DCs express a large variety of receptors, including several classes of pattern-recognition receptors such as Toll-like receptors (TLRs), C-type lectin (CLR) and endocytosis receptors, which are all specialized to recognize and internalize either antibodies, sugars, pathogens, or conserved pathogen-associated molecular patterns (PAMPs).

The capacity of human DC cell surface receptors for antibody mediated antigen targeting approaches is barely investigated. Several studies have used such receptors, e.g. DCIR, DC-SIGN, MMR, and BDCA-2. These studies point out that receptor-mediated antigen targeting using those molecules effects cellular immune responses, even though it is not always clear if rather CD4 or CD8 T cell responses are initiated. Additionally, it remains to be determined how these molecules can be compared to other receptors, such as DEC205, which has become the most commonly used receptor for antigen targeting to human DCs.

To elucidate this question, we chose the human C-type lectin ClecSF6/DCIR/LLIR, whose murine counterparts are DCIR1 and DCIR2. We have generated monoclonal anti-human DCIR antibodies. In order to investigate the antigen targeting properties of the antibody with the highest binding capacity, both the light chain and the heavy chain, carrying the influenza Hemagglutinin protein (HA) at the Fc part, were cloned and produced in 293T cells. We could show that the HA-tagged anti-human DCIR antibody gets internalized into PBMCs within 1 h. To investigate the capacity to induce T cell proliferation, we selectively targeted monocyte-derived DCs with HA-tagged recombinant antibodies to either DEC205 or the DCIR receptor. We could show that targeting HA to DEC205 elicits an IFN-γ production in CD4 as well as inCD8 T cell, whereas targeting HA to DCIR predominantly elicits CD4 T cell responses.

This study demonstrates the differential capacity of hDCIR and hDEC205 to induce recall responses in T cells after antigen targeting to these receptors *in vitro*. It is of great interest, if we could identify further endocytotic receptors which induce different T cell responses.

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P120

Molecular mechanisms of insulin resistance in T-lymphocytes

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T-lymphocytes play a central role in autoimmune diseases like psoriasis. In this chronic inflammatory skin disorder adhesion molecule mediated rolling on the endothelium is prerequisite for extravasation of lymphocytes into the skin, where lymphocytes contribute to the phenotypic consequences of the disease. Activated T-cells and keratinocytes produce proinflammatory cytokines, e.g. tumor-necrosis-factor-alpha (TNF-alpha), interleukin-17 (IL-17), and interleukin-23(IL-23), which also have systemic effects. Thus psoriasis is associated with comorbidities such as hypertension, arteriosclerosis, coronary artery disease and insulin resistance, often leading to the development diabetes mellitus. The molecular mechanisms of insulin resistance have already been intensively examined in classical insulin-responsive tissues (muscle, fat, liver). Whether insulin resistance also occurs in immunological cells and which causal role proinflammatory cytokines might play in this content has not yet been sufficiently investigated.

We aim at providing evidence for T-lymphocyte insulin signalling that can be attenuated *in-vitro* by simulating a psoriatic microenvironment and at identifying the molecular mechanisms involved here. In addition we focus on investigating the impact on T-lymphocyte adhesion molecule expression.

We could show that primary T-lymphocytes as well as TALL-1 cells (a cell line derived from an acute T-cell leukemia) react to insulin stimulation by activating thePI3-Kinase as well as MAP-Kinase pathway. By using different inhibitors the involved signalling molecules could be dissected. Moreover we could show that T-cells can become resistant to insulin by chronic treatment with inflammatory cytokines, such as IL-17, IL-23 and TNF-alpha. In addition insulin signalling influences the expression of adhesion molecules which are dysregulated by inflammatory cytokines contributing to the pathological attachment of lymphocytes to the endothelium.

P121

Generation of Increased Numbers of IgG Plasma Cells in bullous pemphigoid: NC16a specific cells belong to the short-lived Plasma blast population

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Bullous pemphigoid (BP) is characterized by the presence of circulating immunoglobulin G (IgG) autoantibodies directed against the 180 kDa bullous pemphigoid antigen (BP180), also known as collagen XVII. The major immunodominant site of BP180 is located within the noncollagenous (NC16a) domain. In this study we analyzed frequency and phenotype of antigen (NC16a)-specific plasma cells in BP. The enrichment of plasma cells from the peripheral blood of patients and healthy individuals was performed using CD138microbeads. CD138+ plasma cells were stained for different surface molecules as well as intracellularly for immunoglobulin G (icIgG). 50% (+/–8.8%) of the CD138positive cells of BP patients expressed icIgG, while in control persons an icIgG expression was only found in about 24% (+/–7.9%; $P < 0.0001$). Additionally, on average 72.5% (+/–7.0%) of the CD138+ IgG-secreting cells of BP patients showed a high expression for HLA-DR (CD138+HLA-DRhigh) whereas in healthy donors only 50% (+/–9.3%) of these cells expressed this surface marker ($P < 0.0001$). NC16a specific cells were only detected in the HLA-DR high population (1.07% +/–0.66%) and identified as short-lived plasma blasts. In the cell population representing mature long-lived plasma cells expressing low amounts of HLA-DR (CD138+HLA-DRlow), no NC16a specific cells were detected.

These data indicate that in BP a broad autoantigen-independent activation of the plasma cell system occurs. Antigen specific autoreactive plasma cells belong to the short-lived plasma blast subset which could play a role in the immunopathology of BP being prone to remission.

P122

Tumor resident Treg degrade ATP to adenosine via CD39/CD73, resulting in downregulation of CD69 on effector T cells

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Adenosine, which is known to exert immunosuppressive effects in inflammatory processes, is produced via degradation of adenosine triphosphate (ATP) through the ectonucleotidases CD39 and CD73 expressed by CD4+CD25+FoxP3+ regulatory T cells (Treg). As elevated levels of immunosuppressive Treg is frequently observed during tumor growth, our study aimed at investigating the role of Treg-derived adenosine during the growth of murine B16 melanoma.

Mice were s.c. inoculated with 10E5 B16 melanoma cells and sacrificed after the tumors reached a volume of 300–400 mm^3. Spleens, non-draining lymph nodes (ndLN), draining LN (dLN) and tumors were isolated and lymphoid organs of naive mice served as controls. We could show an approximately 100-fold higher concentration of ATP released from freshly isolated and purified B16 melanoma cells, as compared to the cells of the lymphoid organs, using ATP-luminescence assays. Further, analysis of the frequency and the characteristic activation markers expressed by T cells revealed significantly elevated numbers of tumor-resident Treg expressing CD69 in comparison to spleen and LN. These cells also strongly upregulated the ectonucleotidase CD39, the key enzyme for the ATP-to-adenosine degradation. Additionally, the tumor residing effector CD4+ T cells showed low expression of CD69 as compared to the CD4+ T cells in the lymphoid organs, indicating a non-activated phenotype. These data suggest that increased levels of ATP, released by the tumor cells, activate the tumor-resident Treg, followed by CD39mediated degradation of the ATP into adenosine, which subsequently mediates the suppression of tumor specific effector T cells. Thus, the Treg mediated ATP-adenosine turnover may contribute to the immunosuppressive environment of the tumor.

P123

Commensal and pathogenic staphylococci activate different signaling pathways to modulate the innate immune response in human primary keratinocytes

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Human skin is selectively colonized by commensal bacteria, especially by *Staphylococcus epidermidis*, whereas *Staphylococcus aureus* is only rarely found on healthy human skin. The response of keratinocytes to colonization with commensal bacteria and the influence of commensals on the response to pathogens are not well understood. In this study we analyzed microbial and host factors which are involved in the innate immune response of human skin. We examined the expression of antimicrobial peptides and the signalling pathways activated in human primary keratinocytes in response to stimulation with commensal and pathogenic bacteria. Our data indicate that pathogenic staphylococci induce significantly higher expression levels of the antimicrobial peptides HBD-3 and RNase7 compared to the expression induced by commensal staphylococci. Furthermore, whereas skin commensals induce expression of the AMPs HBD-3 and RNase7 in primary human keratinocytes via TLR-2, EGFR- and NFκB-activation, pathogenic staphylococci activate the MAPK- and PI3K/AKT signalling pathways and suppress NFκBactivation. Interestingly, commensal bacteria are able to amplify the innate immune response of human keratinocytes to pathogens by increased induction of AMP expression and abrogation of NFκB suppression suggesting that the two activation pathways can act in a synergistic way. These data indicate that commensal and pathogenic microorganisms evolved specific mechanisms to modulate innate immunity of human skin.

P124

CD36+ leukocytes: common precursors for cutaneous antigen-presenting cells in developing human skin?

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Despite a considerable number of studies, the precise phenotype of skin dendritic cell precursors has not yet been determined in humans. Since these elusive precursors migrate into embryonic skin showing a primitive surface marker profile that subsequently matures into the profile of adult dendritic cells, clues about these precursors can be obtained by the study of their ontogeny. Thus, the expression of selected markers was evaluated on cryostat sections and single cell suspensions of embryonic and fetal skin using confocal laser scanning microscopy and flow cytometry. We found that at 9 weeks estimated gestational age (EGA) the majority ofCD45+HLA-DR+ cells exhibit the scavenger receptor CD36. Immunofluorescence staining of embryonic skin sections locates these CD45+HLA-DR+CD36+ cells predominantly in the dermis, but occasionally also in the epidermis. Furthermore, CD36+HLA-DR- epidermal leukocytes are found until the end of the first trimester. Flow cytometric analysis revealed that CD14 and HLA-DR are expressed on 65.2% (SD ± 9.7, $n = 5$) and 50.4% (SD ± 19.0, $n = 5$) of CD36+ leukocytes in embryonic skin, respectively. Assessment of various C-type lectin receptors on skin immune cells revealed that at the end of the first trimester one third of HLA-DR+CD36+ leukocytes are positive for the mannose receptor CD206. With advancing gestational age the percentage of CD206+CD45+CD36+ cells increases, yet adult-like levels are not reached during in utero development. CD206 expression is restricted to dermal cells during all stages of development. As in adult skin, the expression of CD209/DC–SIGN is exclusively found on dermal CD206+CD1c– cells but never on epidermal langerhans cells during skin development.

Collectively, our data suggest that CD45+CD36+ cells could act as precursors for professional antigen-presenting cells during human skin development.

P125

The role of MyosinIXb in different dendritic cell subtypes and T cells

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The vertebrate motor protein Myosin 9b (Myo9b) is a member of the single-headed class IX myosins. Despite its single motor domain it can move along actin filaments for a long distance without dissociating. Myo9b negatively regulates the small Gprotein Rho that is part of signalling pathways regulated by extracellular factors controlling the organisation of the actin cytoskeleton. In extending lamellipodia, filopodia and membrane ruffles Myo9b is recruited to regions of active actin polymerisation.

Since dendritic cells (DC) as the most important antigen presenting cells in the immune system are characterized by intense cytoskeletal activity, Myo9b deficient mice (Myo9b–/–) were created to analyse the impact of Myo9b on DC functions. We could show that the mobility of bone marrow-derived DC (BMDC), plasmacytoid DC(pDC) and conventional DC (cDC; CD11c+CD8+ and CD11c+CD8–) of Myo9b–/–mice in 3D collagen matrices is decreased compared to that of their wildtype (wt) counterparts. Moreover Myo9b–/– BMDC induced less antigen-specific T cell proliferation while forming prolonged contacts with CD4+ T cells during the initial interaction phase in collagen gels. *In vivo* Myo9b–/– BMDC displayed a reduced migratory capacity when injected into the food pad of C57BL/6 mice.

In further functional studies we wanted to determine whether the loss of Myo9b also has an impact on the mobility of T cells. After isolation of CD4+ T cells from Myo9b–/–and wt mice, we observed a reduced velocity as well as an impaired proliferation of knock out cells in a mixed lymphocyte reaction with allogeneic BMDC. Taken together our data suggest that Myosin9b is of crucial importance for the migration of DC and T cells and that the loss of Myo9b results in reduced immune responses.

P126

Gene gun immunization with replicase-based expression plasmids engages langerin-expressing cells for enhanced induction of cytotoxic T-cell responses

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Particle-bombardment of the skin with plasmid DNA using the gene gun can induce Ag-specific CD8+ cytotoxic T-cells which are important for protection against viral diseases and cancer. Here we further investigated the use of plasmid DNA encoding a Sindbis virus replicon to increase the efficacy this genetic immunization approach in mice. We found that particle-mediated direct transfection of the skin with replicase-based expression plasmids led to significantly stronger expansion and cytotoxic activity of adoptively transferred OVA-specific TCR-transgenic CD8+ T-cells when compared with conventional expression plasmids. Using novel diphtheria toxin receptor-based experimental systems we found that conditional depletion of langerin-expressing cells, which include epidermal Langerhans cells and a subset of dermal dendritic cells, impaired the induction of cytotoxic T-cells only with replicase-based but not with conventional expression plasmids. In contrast, conditional depletion of CD11c+ cells revealed that both immunization strategies critically required the presence of Ag-presenting dendritic cells for the stimulation ofCD8+ T-cells. Taken together, our experimental results provide evidence that cutaneous gene gun immunization with replicase-based expression plasmids engages langerin+ dendritic cells in the skin to enhance the induction of cytotoxic cellular immunity. We are currently investigating the tumor immuno surveillance function of gene gun-stimulated CD8+ T-cells using adoptively transferred lymphocytes derived from pmel-1 TCR transgenic mice. Pmel CD8+ T-cells recognize the gp100 antigen, a melanosomal protein naturally expressed by melanocytes and transplanted B16 melanoma cells in the skin.

P127

Oncolytic adenoviruses for combined immuno-virotherapy of malignant melanoma

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Virotherapy is a promising new tool for treatment of cancer by tumor-specific viral replication, cell lysis and spread of progeny viruses. Oncolytic Adenoviruses are leading oncolytic agents for virotherapy for which several clinical trials have demonstrated proof of principle and a favorable safety profile. However, adeno viraloncolysis needs to be improved to achieve therapeutic benefit in the clinic. Towards this end our work aims at combining adenoviral oncolysis with the induction of systemic and sustained anti-tumor immunity by the viral tumor cell lysate. Here we focus on the investigation and manipulation of immune cell recruitment by adenoviral oncolysis.

We engineered a melanoma-targeted oncolytic adenovirus (Ad5/3.2×Tyr) that showed a strongly enhanced infection rate of freshly isolated melanoma cells and a block of their replication cycle in non-melanoma cells, thus establishing tumor-selectivity and efficacy.

So far it is not known if a specific anti-tumor immune response could be induced by oncolytic adenoviruses in humans. Therefore, we analyzed the profile of chemokines expressed by human melanoma cells before and after infection with Ad5/3.2×Tyr on mRNA and protein level. To investigate the three-dimensional viral spreading and impact of the tumor-microenvironment on production of immune modulators, we established living precision-cut tissue slices of melanoma biopsies. Moreover, we performed migration assays to analyze the effect of oncolysates on endothelial cell-dependent and -independent recruitment of dencritic cells. Once recruited, these should then phagocytose oncolysate to activate systemic anti-tumor immunity. Our data showed that infection of cultured low passage melanoma cells and established melanoma cell lines with Ad5/3.2×Tyr does not induce significant changes in chemokine production and immune cell recruitment in comparison to non-infected cells. Furthermore, we could show that human CCL5 complements oncolytic adenoviral oncolysate for recruitment of immature dendritic cells. Additional data with endothelial cell-dependent migration and primary biopsy material will be presented. Our results indicate that anti-tumor immune activation by adenoviral oncolysis of melanoma would benefit from targeted manipulation of immune cell recruitment. Towards this end, we currently develop optimized oncolytic adenoviruses expressing human CCL5 to induce increased tumor infiltration by immature dendritic cells. We hypothesize that infection of melanomas with such genetically engineered oncolytic adenoviruses improves the clinical benefit of virotherapy.

P128

Regulatory function of Cytip in mouse Dendritic cells during contact hypersensitivity reaction

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Cytip (Cytohesin 1 Interacting Protein) was suggested to be involved in the immunological synapse formation and duration of T cell and dendritic cells (DC) contact.

To investigate the role of Cytip in mouse dendritic cells, we first compared the number of different DC subpopulation in skin draining lymph nodes in wild type and Cytip ko mice. No difference could be found regarding Langerhans cells, dermal langerin+ DC and dermal dendritic cells between Cytip ko mice and their wildtype counterparts.

To further investigate the role of Cytip in DC we used ovalbumin loaded BMDC (bone marrow derived dendritic cells) as antigen presenting cells (APC) in a mixed lymphocyte reaction (MLR) with either OT-I or OT-II T cells. In both MLRs we measured higher proliferation when BMDC generated from Cytip ko mice were used as APC. We also added natural occurring regulatory T cells (nTregs) in different concentrations to these MLR and observed less suppressive capacity when Cytip koDC were utilized as antigen presenting cells.

To rule out the function of Cytip in DC *in vivo*, we used TNBS modified bone marrow derived DC from wildtype and ko mice and injected them intradermally into wildtype recipients. After 5 days mice were challenged with 1% TNCB on both sides of the ear, and ear swelling was measured. In wildtype mice sensitized with Cytip ko BMDC, ear swelling reaction was much more pronounced than in those mice treated with wildtype BMDC. In addition to these results we explored also the role of Cytip in DC during the challenge phase of contact hypersensitivity reaction. For this we sensitized wildtype mice with 1% TNCB on the shaved abdomen and transferred spleen cells into wildtype and Cytip ko mice 5 days after sensitization. Mice were challenged with TNCB 2 h after spleen cell transfer and again ear swelling reaction was measured. Also under these conditions, we found higher ear swelling in Cytip ko mice.

These results indicate a regulatory function of Cytip in mouse DC although the molecular mechanism is still elusive.

P129

Autoantibodies in anti-p200/laminin γ1 pemphigoid: development of a sensitive and specific detection system using the C-terminal portion of laminin ?γ1

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Anti-laminin γ1 pemphigoid is an autoimmune subepidermal blistering disease which was first described as anti-p200 pemphigoid in 1996. This disease is characterized by autoantibodies against a 200 kDa protein (p200) of the dermal-epidermal junction. Recently, the laminin γ1 chain has been identified as the target antigen and the C-terminus of laminin γ1 was described as an immunodominant region of this protein. Diagnosis of this disease requires detection of serum IgG binding to the dermal side of 1 M salt-split skin by indirect immunofluorescence microscopy and labelling of a 200 kDa protein by immunoblotting with extract of human dermis. However, so far, the diagnosis depended on the quality of the dermal extract. Here, we report on a sensitive and specific Western blot analysis for the diagnosis of anti-p200/laminin γ1pemphigoid using a recombinant peptide. The C-terminal fragment of human laminin γ1 (hLAMC1-cterm; amino acids 1364–1609) was expressed in *E. coli*. After purification of hLAMC1-cterm by immobilized metal ion affinity chromatography, the peptide was used for Western blotting. Serum reactivity with hLAMC1-cterm was observed in 25 of 29 (86%) patients with anti-p200/laminin γ1 pemphigoid, where as sera from only 1 of 30 (3.3%) bullous pemphigoid patients and none of 40 healthy volunteers showed reactivity. Western blotting of the C-terminal fragment of laminin γ1 will considerably improve the diagnosis of anti-p200/laminin γ1 pemphigoid, an as yet under diagnosed entity.

P130

Osteopontin is a regulator of effector T cell functions during the elicitation phase of allergic contact hypersensitivity

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The phosphoglycoprotein Osteopontin (OPN) has chemotactic and Th1 cytokine functions and in various models is essential for robust T cell mediated immunity. In allergic contact hypersensitivity we found that memory T cells are a major source of OPN. OPN secretion is induced on the mRNA and protein level in antigen specific CD4+ and CD8+ T cells of TNCB sensitized mice. To prove the *in vivo* relevance of OPN expression, OPN wild type and OPN null mice were compared in their CHS response to TNCB and indeed OPN null mice had reduced acute and chronic ear swelling response. To further investigate the role of T cell expressed OPN for the effector phase of CHS, CFSE labelled T cells from wild type or OPN null mice we retransferred into OPN wild type RAG2–/– mice that were then sensitized with TNCB and challenged on day 5. In control animals OPN null CD8+ but not OPN null CD4+ T cells showed an increased homoeostatic proliferation in skin draining lymph nodes. While skin antigen challenge strongly induce fast proliferation of OPN wild type CD8+T cells, OPN null CD8+ T cells largely remained in the homeostatic proliferation state. In contrast OPN deficiency did not alter proliferation of CD4+ T cells. When investigating the influx of T cells into the challenged ear skin, the mice that had received the OPN null T cells showed impaired influx of both CD4 and CD8 cells into the elicitation site. Our findings indicate that OPN produced by antigen-specific T cells is importantly involved in regulating the proliferation and the chemotactic attraction of effector T cells during the effector phase of CHS.

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Regulatory monocytes contribute to control inflammatory disorders and autoimmunity

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Glucocorticoids (GC) induce a novel phenotype in monocytes that confers regulation to cells of the adaptive immune system, especially to T cells. In a murine model of chronic inflammatory colitis we demonstrate that these regulatory monocytes (Mregs)are of clinical relevance to reduce inflammation and weight loss in the animals. Using their surface molecules PD- L1 and CD124 Mregs regulate CD4 and CD8 T cells. Effector T cells are diminished in their capacity to produce pro- inflammatory cytokines IFN-γ and IL-17 when they have contact to Mregs. Physiologically, Mregs differentiate from blood monocytes when glucocorticoid levels are elevated e.g. by stress or even systemic bacterial infection, and thus are one mechanism of the immune system to down- regulate chronic inflammatory reactions. In conclusion, Mreg potentially can be used as targets for immune intervention that aims to downregulate and control inflammatory disorders.

P132

Interleukin-17 acts as unspecific amplifier of allergic skin diseases

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Interleukin (IL)-17 is a pro-inflammatory tissue-instructing cytokine. In this study, we characterize distinct IL-17 producing T cell populations infiltrating the skin during acute atopic eczema (AE, $n = 4$) and allergic contact dermatitis (ACD, $n = 4$) reactions and describe multiple roles of IL-17 in the amplification of the inflammatory response. Among T cell cones derived from lesional skin (total = 144 from AE, 320 from ACD), about 15–20% secreted IL-17 after stimulation with PMA/Ionomycin. IL-17+ T cell clones could be categorized as Th17, Th1/IL-17 and Th2/IL-17. Notably and in contrast to other helper T cells, pure Th17 cells were not specific for the causative allergen (Der p 1 for AE, Nickel sulfate for ACD). To elucidate functional aspects of T cell-derived IL-17, we stimulated primary human keratinocytes with T cell supernatants and recombinant IL-17, respectively. T cell-derived IL-17 elicited a strong production of antimicrobial peptides and IL-8, thus promoting an innate immune response of the epithelium. Interestingly, IL-17 also promoted the IFN-γ mediated upregulation of ICAM-1 on keratinocytes. This upregulation resulted in enhanced cell-to-cell contact of cytotoxic T cells and keratinocytes, which led to keratinocyte apoptosis even in the absence of the specific allergen. In conclusion, our data demonstrate that IL-17 is a central pro-inflammatory mediator in the skin microenvironment – by amplifiying a non-specific cytotoxic cascade it causes severed and sustained cutaneous inflammatory reaction.

P133

Syndecan 1 and Syndecan 4 affect the *in vivo* migration of dendritic cells

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The antigen-dependent activation and migration of Dendritic Cells (DC) in peripheral tissues is closely associated with interactions with the Extracellular Matrix (ECM).Syndecans (SDC) are trans membrane proteoglycans with heparin sulfate side chains. SDC act as integrin co-receptors and sequester extracellular signals like cytokines, thereby affecting cell migration. For the further role of SDC1 and SDC4 we investigated the *in vivo* DC migration in wild-type (WT) mice (CD57/Bl6) compared to SDC deficient mice (SDC1–/– and SDC4–/–).

Analysis of naïve lymph nodes revealed a decreased CD11c positive DC count inSDC4–/– compared to WT mice whereas SDC1–/– reveal normal counts of DC in lymph node Following Tetramethyl-rhodamine-iso-thiocyanate (TRITC) painting of the skin DC migration to draining lymph nodes was monitored for 12 h, 24 h, and 96 h by flow-cytometric identification of CD11c and TRITC positive cells. A slightly enhanced migration of SDC1–/– DC and a delayed migration of SDC4–/– DC were observed. This effect indicates the importance of the SDC function in cell migration and is consistent with the results of prior *in vitro* motility assays.

P134 (V19)

Skin mast cell populations do not recover in adult mice after induced ablation of mature mast cells but return under inflammatory conditions

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Mast cells reside in virtually all vascularized tissues. Apart from their role in type I hypersensitivity responses, they were shown to be important initiators of innate immune responses as well as modulators of adaptive immunity.

Mast cells originate from hematopoietic precursors in the bone marrow. Committed mast cell progenitors are released into the blood and circulate until they extravasate into the tissues where they finally differentiate into mature mast cells. These mast cell progenitors can be detected in the blood of the mouse embryo during a short time window (E15.5), but not at later stages of ontogeny. The prevailing belief is that, throughout life, peripheral mast cell populations are continuously replenished by hematopoietic progenitors.

By crossing A-Mcpt5-Cre mice, which delete loxP-flanked DNA selectively in mature connective tissue mast cells (CTMC), to iDTR mice, we established a mouse model that allows for efficient inducible depletion of CTMC in adult mice. In the A-Mcpt5-CreiDTR double positive mice, mast cells are rendered diphtheria toxin (DT)-sensitive due to Cre-mediated expression of the high affinity DT receptor. Intraperitoneal DT injections resulted in efficient skin mast cell depletion. Interestingly, skin mast cell numbers recovered with a very slow kinetic, reaching only 15% of original numbers even 1 year after depletion. Real-time PCR analysis of total RNA isolated from mast cell-depleted skin revealed that the expression of SCF (stem cell factor), the most important mast cell growth factor, is significantly upregulated after mast cell depletion. However, this upregulation of SCF did not appear to sustainably affect mast cell repopulation in the skin. In contrast, after induction of skin inflammation by TPA application, mast cells again accumulated in mast cell-depleted skin. Our results indicate that peripheral mast cell populations are not or only very slowly replenished by hematopoietic precursors and that the steady state homeostasis and the recruitment of mast cells under inflammatory conditions are regulated by different mechanisms.

P135

Pharmacological inhibition, but not a lack of expression, of tumor necrosis factor alpha improves experimental epidermolysis bullosa acquisita

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Epidermolysis bullosa acquisita (EBA) is a difficult-treat sub epidermal autoimmune blistering skin disease with circulating and tissue bound ant-type VII collagen antibodies. Different reports have indicated an increased expression of tumor necrosis factor α (TNFα) in serum and blister fluid of patients with subepidermal autoimmune blistering skin disease. Furthermore, successful anti-TNFα treatment has been reported for individual patients. Here, we show that induction of experimental EBA in mice, by repeated injection of rabbit-anti mouse type VII collagen (mCOLVII) antibodies, led to an increased expression of TNFα in the skin as determined by PCR and immunohistochemistry. To investigate if the increased TNFα expression is of functional relevance in experimental EBA, rabbit-anti-mCOLVIIIgG was injected into TNFα-deficient mice. No difference in disease severity was observed compared to wild type controls. Subsequently, we inhibited TNFα function using a pharmacological approach. The soluble TNFα receptor fusion protein etanercept (Enbrel®) and a monoclonal antibody (mAb) to murine TNFα were studied for their impact on experimental EBA. In brief, the Enbrel or mAb to murine TNFα treatment was started 2 days prior to the initial rabbit-anti-mCOLVII IgG injection. Interestingly, mice receiving either of these two treatments showed significantly milder disease than controls.

Immunohistochemical staining demonstrated reduction of macrophages in lesional skin particularly in mice treated with the mAb to murine TNFα, compared to controls. In conclusion, the increased expression of TNFα in expreimental EBA is of functional relevance, as both etanercept and anti-TNFα antibodies impaired the induction of experimental EBA. The different results observed in TNFα-deficient mice compared to the pharmacological inhibition studies might be explained by a compensatory mechanism in the deficient mice. In future studies, the effect of TNFα inhibition in a therapeutic setting will be investigated to closely mimic patients' clinical situation and to fully understand this cytokine's role in subepidermal autoimmune blistering skin diseases.

P136

Monitoring of vaccine-specific cytolytic, helper and regulatory T cells in melanoma patients after vaccination with autologous antigen-loaded dendritic cells

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Cancer vaccines have been shown to induce tumor-specific CD8+ cytolytic T cells (CTL) in the blood of cancer patients. However, clinical outcome often remains poor. This ''cancer vaccine paradox'' can nowadays be readily explained by escape and immune-regulatory mechanisms. It has been suspected, that cancer vaccines of different types might not only induce the desired CTL response but simultaneously also expand antigen-specific regulatory T cells (Treg), thus counteracting productive immune responses and preventing clinical efficacy of cancer vaccines. Of note is that it has been claimed that mature dendritic cells (DC) are not only expanding antigen-specific helper T cells but also the disadvantageous Tregs. The induction of antigen-specific Tregs by DC and other vaccines has, however, not yet been studied systematically by advanced immunomonitoring techniques.

As a first approach to discover the nature of elevated Treg frequencies in vaccinated cancer patients we analyzed their specificity for the vaccine or other tumor antigens.10 color flow cytometry and MHC-Class I and MHC-Class II multimeters were used to determine the cytolytic potential of vaccine-specific CD8+ T cells and the regulatory phenotype of vaccine-specific CD4+ T cells upon vaccination of melanoma patients with i) peptides ii) peptide loaded dendritic cells (DC) or iii) DC transfected with RNA coding for different tumor-associated antigens. Our first results demonstrate that DC loaded with peptides induce vaccine-specific CD4+ T cells without regulatory phenotype at least during the course of vaccinations (up to 2 years). Interestingly, this was also the case in patients who upon disease progression presented a rising frequency of Tregs with unknown specificity.

In contrast to common belief our data so far do not support the notion that tumor vaccination with autologous mature dendritic cells bears the risk to induce immuno-suppressive regulatory T cells.

P137

Comparative studies of *in vitro* generated mast cell from different origin

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Mast cells (MC) are not only key effector cells in immediate type I reactions but also play a crucial role in innate and adaptive immunity. Consequently, MC investigations *in vitro* and *in vivo* steadily increase. As it is difficult to obtain primary MC in large numbers, it is well accepted to work with *in vitro* generated MC. The classical way to generate MC is from bone marrow (BMMC), alternatively MC can be obtained from fetal skin (FSMC) or from fetal liver (FLMC). As these cultures may differ and analyses about the influence of the MC source on quantity and quality of the *in vitro* induced cells have not been done, we established the side-by-side culture and generation of these MC types and performed comparative studies in respect of morphology, surface marker expression, and release of mediators in immediate, intermediate, and late responses. While all three cell types were clearly identified as MC by different means, we found several differences concerning proliferation, lifespan and maturation. FSMC seem to be more mature and/or more responsive, as they show a higher granule content and mediator release but lower proliferation capacity than BMMC and FLMC. Functional analyses with IgE-DNP revealed for all three MC types unequivocal immediate Ca2+-influx and identical sustained activation as detected by patch-clamp experiments for the K-channel SK4. Finally, investigating the innate immune response by TLR stimulation we found for all three MC types the same results for several parameters on RNA and protein level except for IL-10, which was highest for BMMC and low or undetectable for FLMC and FSMC, respectively. Most interestingly, analysis of the temporal dynamics of EYFP-expression dependent on the MC-specific Mcpt5 promoter resulted in two subpopulations only for BMMC but not for FLMC and FSMC cultures indicating incomplete or delayed maturation of BMMC.

These investigations are most helpful to generate large quantities of well characterized MC, but also indicate that there may be peculiar differences that need to be addressed especially when MC functions as immune regulators are studied.

P138

TLR2 dependent cutaneous inflammation is based on IL-4 mediated suppression of IL-10

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Atopic dermatitis (AD) is a T cell mediated inflammatory skin disease and associated with Th2 immune responses. Inflamed AD skin is highly susceptible to colonization and infection with the gram-positive bacteria *Staphylococcus aureus*. Innate immune sensing of bacteria has been well characterized in the past decade and gram-positive bacteria provide potent TLR2 ligands. However, the role of innate immune sensing for AD inflammation remained enigmatic. Moreover, the impact of the early predominance of Th2 derived IL-4 on innate immune sensing and ongoing inflammation is still unclear. To this end we investigated innate immune sensing of *S.aureus* in AD by analyzing consequences of TLR2 activation in the context of cardinal features of atopic inflammation. We show here that different TLR2 ligands stimulate dendritic cells (DC) to produce IL-12p40, IL-12p70 and IL-23 as well asIL-10. In addition, TLR2 ligands also co-stimulate Th cells and amplify T cell proliferation and T cell cytokine production. In models of Th2 cell mediated cutaneous inflammation a direct and long lasting amplification of cutaneous inflammation byTLR2 ligands could be demonstrated. Adoptive transfer experiments revealed that accessory cells but not T cells are responsible for TLR2 mediated cutaneous inflammation *in vivo*. Most importantly, we identify IL-10 as key regulator of TLR2mediated inflammation. IL-4 shuts down TLR2 mediated IL-10 production by >90%in DC and mast cells. Moreover, TLR2 mediated cutaneous inflammation is most pronounced in the absence of IL-10. This indicates that IL-4 mediated suppression ofIL-10 maybe crucial for the initiation of chronic inflammation by Th2 cells.

P139

CD40 signalling in dendritic cells: Balance between immunity and tolerance

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DC are key players in the induction of immunity and tolerance. The present study was designed to characterize the role of the CD40-CD40L pathway for differentiation and functional maturation of human immature DC. Therefore, immature DC were stimulated through anti-CD40 antibodies or adenoviral CD40L transfection. Resulting DC (CD40-DC) were analyzed concerning their phenotype, cytokine profile and T cell stimulatory capacity. CD40-DC exhibited a largely comparable phenotype to fully mature immunostimulatory DC with an upregulation of costimulatory molecules and an increased production of IL-12p70. However, their T cell stimulatory properties were markedly impaired. Stimulation of CD4+ or CD8+ T cells with CD40-DC resulted in the induction of IL-10-producing regulatory T cells. This phenomenon was shown to be due to an enhanced IL-10 production of CD40-DC and stabilization of the IL-10 receptor expression on activated T cells. Furthermore, OX40L, a molecule which strongly inhibits IL-10 production of mature DC was not expressed by CD40-DC. Nevertheless, additional stimulation with proinflammatory cytokines induced the differentiation of CD40-DC into immunostimulatory DC and restored their T cell stimulatory properties. These data demonstrate that CD40 signalling in the absence of inflammatory signals induce the differentiation of immature DC into tolerogenic DC with strong immunosuppressive properties.

P140

Antihistamines as immune suppressants

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Histamine is typically thought of as a mediator of allergic responses, and antihistamines are therefore typically used to treat allergic reactions of the conjunctival or nasal mucosa as well as the skin. However, mast cells store vast amounts of histamines in all tissues, not just mucosa and skin. Also, H1 receptors are expressed ubiquitously and on various cell types, including leukocytes. This suggests a role for histamine as a general immune modulator, not only in allergy. In various mouse models we have therefore analyzed the effect of antihistamines on susceptibility to viral and bacterial infections, on alloreactive T-cell responsiveness, psoriasis, diabetes and septic shock. To our surprise we found that certain antihistamines showed immune suppressive effects that were comparable in strength to Ciclosporin A and Dexamethasone. Mice medicated with antihistamines succumbed to lethal listeriosis, and could no longer eliminate lymphocytic-choriomeningitis virus. These immune suppressive effects could also be used to prolong survival of solid organ transplants, or in the treatment of psoriasis and of septic shock. *In vitro* studies suggest that certain antihistamines have similar effects on the human immune system and that these effects are achievable with therapeutic doses of antihistamines. In conclusion, these findings question the wide spread and sometimes uncritical use of antihistamines, but also suggest a number of new therapeutic indications for antihistamines.

P141

Differential expression of antimicrobial peptides in the vestibulum nasi and the secretion in nasal fluids

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The nose is known to be a reservoir for multiple bacteria especially *S. aureus* and needs in particular an efficiently working system to control bacterial growth. Antimicrobial peptides (AMP) are naturally produced human antibiotics, which play an important role in first line defense against invading microorganisms. Aim of our study was to investigate for the first time systematically the expression of several AMP (psoriasin (S100A7), RNase 7, LL-37, human beta defensin (hBD) -2 and -3 of healthy individuals in the vestibulum nasi and the secretion in nasal fluids.

Expression of psoriasin, RNase 7, LL-37, hBD-2 und -3, had been determined by immunhistochemistry (IHC) in six samples derived from healthy nasal mucosa of the vestibulum nasi. IHC-scoring for quantitative analysis was performed by two independent investigators, scoring every epidermal layer in every visual field, differentiate between faint, intermediate and high immunreactivity. AMP secretion was analyzed in standardized nasal swab fluids of 20 healthy individuals (14f/6m;age 26–55) using specific sandwich ELISA for psoriasin, RNase 7, LL-37, hBD-2 und-3.

Immunhistochemistry and IHC-scoring revealed strong expression of psoriasin and RNase 7, whereas hBD-3 was only faintly visible in the upper layers of the transitional epithelium. LL-37 and hBD-2 were not detectable.

Secretion levels of the in keratinocytes constitutively expressed AMP psoriasin and RNase 7 in nasal swab fluids differed only slightly, but were highest of all detected AMP. Inducible AMP such as LL-37, hBD-3 and hBD-2 showed secretion levels lower than psoriasin and RNase 7 but were all detectable.

Taken together, these results suggest that AMP play an important role in first line defense of the nose and to control bacterial growth. Future studies have to show if an impaired AMP expression and secretion may play a role in *S. aureus* colonization of the nose especially in patients with recurrent infections caused by such colonization.

P142

Resveratrol modulates UV-induced regulatory T cells via IL-10 and TGF-β

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Ultraviolet radiation (UV) suppresses the immune system in an antigen-specific fashion via induction of regulatory T cells (Treg). The mechanisms underlying both the induction and the activity of UV-Treg are still unclear. Recently, it was shown that resveratrol, a nonflavinoid polyphenolic compound found in many plants, which exerts antioxidant and anti-inflammatory properties, reverses UV-induced immuno suppression and -tolerance. It was shown that UV-induced Treg act via interleukin-10 (IL-10). Furthermore, transforming growth factor-β (TGF-β) is known to be important for the development and function of UV-induced Treg. Since the effect of UV-induced Treg was inhibited by *in vitro* incubation with resveratrol, we studied whether resveratrol is able to modulate the release of these cytokines. To address this issue, bulk cells from lymph nodes and spleens obtained from UV-irradiated and DNFB-sensitized C57BL/6 mice, were coupled *in vitro* with DNBS, the water soluble analog of DNFB, and stimulated with resveratrol (25, 50, 75 μM).48 h later supernatants were harvested and cytokine levels of TGF-β and IL-10 were measured by ELISA. T lymphocytes obtained from UV-irradiated mice produced very high amounts of IL-10, confirming successful stimulation of UV-Treg. Upon treatment of T lymphocytes with resveratrol a pronounced dose-dependent reduction of IL-10 protein was measured. Similar effects were observed when levels of TGF-?β protein were evaluated. These results might explain the antagonizing effect of resveratrol on UV-induced Treg and UV-induced immunotolerance.

P143 (V17)

Targeted depletion of IL-23 by IL-4 *in vivo* impairs T cell mediated delayed-type hypersensitivity

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Over decades, the scientific debate on the pathogenesis of the T-cell mediated delayed-type hypersensitivity reactions (DTHR) was focused on Th1/Tc1 andTh2/Tc2 cells. IFN-γ producing Th1 cells and Tc1 were considered to be the primary effector cells and IL-4 producing Th2 cells to be the counter regulator of DTHR responses. Indeed, systemic IL-4 treatment was shown to reverse established DTHR. More recently, experimental evidence on the role of Th17/Tc17 in DTHR increased, but the role of the Th17 sustaining IL-23, especially in the context of successful IL-4treatment, remained elusive.

To address these questions, we used a model of TNCB-induced DTHR in C57Bl6mice. TNCB challenge in sensitized mice induced pronounced ear swelling and skin inflammation characterized by epidermal hyperplasia, subcorneal neutrophilicin filtrates and prominent papillary blood vessels. Interestingly, the inflammatory infiltrate was dominated by both Th17 cell-associated IL-23 and IL-17 cytokine expression. Systemic IL-4 treatment of these mice resulted in significant reduction of ear swelling and normalized skin morphology. Moreover, we found that IL-4 therapy selectively suppressed cutaneous IL23A but not IL12A mRNA expression. In agreement with the critical role of IL-23 in inducing and sustaining Th17 responses, efficient IL-4 treatment not only suppressed lesional IL23A but also IL17A mRNA in mouse ears. To further analyze this unique and selective suppression of IL-23 byIL-4, mouse bone marrow-derived dendritic cells (mBMDC) were allowed to mature in an IL-4 dominated milieu. In these mBMDC IL-4 suppressed IL-23 to 40% or less of its original levels while promoting 200% up regulation of IL-12. Protein production was regulated at the transcriptional level, with IL23A and IL12A mRNA suppressed and increased respectively. Unraveling the molecular mechanism behind this phenomenon, we found that IL-4 exerts its regulatory effect over both IL-23 and IL-12 in mBMDC in a STAT6 dependent manner. Our findings not only assign cutaneous DTHR as IL-23 and Th17 dependent, we also discovered a novel and unique strategy to selectively target IL-23, which might be beneficial and serve as "proof-of-concept" for the whole group of Th17-associated T-cell mediated diseases such as psoriasis, Crohn disease, rheumatoid arthritis or multiple sclerosis.

P144

Comparative analyses of CD4+ and CD8+ T cells in melanoma patients after high- versus intermediate-dose interferon-alpha therapy

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The effects of interferon-alpha (IFN-α) therapy in melanoma patients have been explored in multiple clinical trials. However, dosage and duration of the regimens are still a matter of discussion. In this study, we analysed the impact of IFN-α on CD4+ and CD8+ T cell populations before and during intermediate-dose in stage Ib-II versus high-dose therapy in stage III melanoma patients. After 4 weeks of IFN-α treatment, significant increases in the percentage of CD4+ T cells were observed in high-dose, but not during intermediate-dose therapy. This was accompanied by an enhanced expression of the early activation marker CD69 and the adhesion molecule CD62L in CD4+ T cells. After high-dose regime, analysis of CD4+CD25 high T cells, considered as naturally occurring regulatory T cells (nTregs), revealed a trend of an elevated percentage of this T cell subset but diminished expression of FoxP3. In these patients, the expression of CD69, the CD45RA molecule, the chemokine receptor CCR7 and the adhesion molecule CD62L were significantly upregulated inCD4+CD25high T cells. In contrast, after intermediate-dose therapy no changes in the rate of CD4+CD25high T cells and the expression of FoxP3 were found. Analyses of CD8+ T cells did not reveal any alteration in the percentage of the T cell subset after intermediate- or high-dose IFN-α therapy, but IFN-a treatment induced a significantly enhanced expression of leukocyte adhesion molecule CD62L in CD8+ T cells in both therapy regimens, whereas activation marker CD69 significantly augmented during high-dose therapy. Taken together, our data indicate that pronounced cellular immune responses are induced by high-dose IFN-a therapy as compared to the intermediate-dose regime, resulting in activation of CD4+ and CD8+T cell subsets and in elevated rates of CD4+CD25 high T cells representing activated effector/memory CD4+ T cells.

P145

The role of FcRs in the activation of human neutrophils in *ex vivo* and *in vitro* models of bullous pemphigoid

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Bullous pemphigoid (BP) is an autoimmune bullous disease associated with autoantibodies to the hemidesmosomal proteins. It is characterized by chronic inflammation and subepidermal blister formation in the skin. Activated neutrophils and their release products, e.g. oxygen radicals and granule constituents, play an essential role in the pathogenesis of the disease. Neutrophil activation is triggered by the binding of FcγRs to immune complexes. However, the role of individual Fcγ receptors in this process is still not clear. In this study, we investigated the role of Fcγ receptors evaluated the direct pathological consequences of neutrophil activation in terms of dermal-epidermal separation in an *ex vivo* model of BP. Furthermore, to gain some mechanism underlying the role of Fcγ receptors, we established an *in vitro* assay and used it to study the role of Fcλ receptors in the activation of neutrophils in terms of neutrophil degranulation, oxygen radical formation and morphological changes. Our results demonstrated that both, FcγRII and FcγRIII, are essential for dermal-epidermal separation in the *ex vivo* model. Using the *in vitro* assay, we showed that FcγRII and FcλRIII are involved in human neutrophil degranulation, reactive oxygen species production and neutrophils spreading under the stimulation of immobilized immune complex. These results indicate that FcγRII and FcγRIII play essential role in the pathogenesis of BP.

P146

OX40/OX40 ligand interaction enhances the development of autoantibody mediated epidermolysis bullosa acquisita

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Epidermolysis bullosa acquisita (EBA) is a severe bullous skin disease mediated by autoantibodies against type VII collagen, a major component of anchoring fibrils of the skin. Different mechanisms leading to autoantibody-induced tissue damage in the skin of EBA patients have been identified. However, events leading to the loss of tolerance to type VII collagen remain largely unknown. We have recently shown that T cells are required for the induction of experimental EBA by immunization of mice with recombinant murine type VII collagen. OX40 and OX40 ligand (OX40L) are members of the TNF receptor/TNF super family, and their interaction promotes division and survival of T cells. We therefore hypothesized that OX40/OX40Linteractions may contribute to the development of experimental EBA. Indeed, treatment of SJL mice with an agonistic OX40 antibody followed by immunization with recombinant murine type VII collagen, enhanced EBA. This was correlated with the increase of CD25+OX40+ activated CD4+ cells. Interestingly, however, engagement of OX40L by a blocking antibody also exacerbated the blistering phenotype of the mice. The blockade of OX40L was accompanied by an inhibition of T cell activation and by an increased influx of OX40L expressing dendritic cells and macrophages into the skin. Our results suggest that blocking of OX40L may exacerbate blistering in experimental EBA by an increased activation of phagocytic cells, like dendritic cells, neutrophils, and monocytes/macrophages. This is in line with the observation that presence and activation of neutrophils in the skin of EBA mice are prerequisites for blister formation.

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Association of IgE - BP autoantibodies (BP180 und 230) and total IgE levels inpatients with and without bullous pemphigoid (BP)

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Introduction: IgE-BP autoantibodies have been known for several years in patients with bullous pemphigoid (BP). Their relation in pathogenesis and clinical expression are likewise discussed. In contrast to IgG-BP autoantibodies (up to 95% for BP 180), IgE-BP autoantibodies are not detectable in all patients with BP (dependent on literature: 22–67%).

Objectives: Association of IgE - BP autoantibodies (BP180 und 230) and total IgE levels in patients with and without BP. We were particularly interested to find out if inpatients without bullous pemphigoid IgE-autoantibodies would be present. Additionally, IgG- and IgE-autoantibodies were compared in BP and non-BP patients.

Methods: Sera obtained from 31 BP patients between 2006 and 2008 as well as 31 sera from control patients (assumed but no confirmed BP) were measured for serum IgG and IgE levels of anti-BP180 (NC16A) and anti-BP230 antibodies (ELISA MBL®) as well as total IgE levels (Phadia®).

Results: In 30 of 31 patients with BP (96.8%), IgG anti-BP180 antibodies (Abs) and in18 of 31 patients with BP (58.1%), IgG anti-BP230 Abs were detectable. IgEanti-BP180 Abs were found in 21 (67.7%) of 31 patients with IgG anti-BP180 Abs. Seventeen (94.4%) of 18 patients with IgG anti-BP230 Abs expressed IgEanti-BP230 Abs. Striking in this subgroup was that in 5 patients without IgGanti-BP230 Abs IgE Abs were detected. Altogether 21 (67.7%) of 31 patients with BP were positive for IgE anti-BP230 Abs.

The total IgE levels were divided into a group >500 and <500 kU/l.

Fourteen of the patients with BP had a total IgE level >500 (536–5000 kU/l) and 17 patients a total IgE level <500 (2.3–392 kU/l). In the control group 16 patients expressed a total IgE serum level >500 (523–5000 kU/l) and 15 < 500 (2.0–419 kU/l).In the subgroup total IgE level >500 kU/l of patients with BP 82.4% positive IgEanti-BP180 Abs (subgroup total IgE level <500 kU/l: 50% IgE anti-BP180 Abs) and100% positive IgE anti-BP230 Abs (subgroup total IgE level <500 kU/l: 35.7% IgEanti-BP230 Abs) were found. There was a significant association of IgE anti-BP230Abs with total IgE level >500 kU/l in BP ($P < 0.01$) but not for IgE anti-BP180 Abs($P = 0.121$).

In the total IgE level >500 kU/l subgroup of control patients without BP 6.3% expressed IgE anti-BP180 Abs (subgroup total IgE level <500 kU/l: 6.6% IgEanti-BP180 Abs) and 25% positive IgE anti-BP230 Abs (subgroup total IgE level <500 kU/l: 0.0% IgE anti-BP230 Abs). There was no significant association of IgEanti-BP230 Abs ($P = 0.101$) and IgE anti-BP180 Abs ($P = 1.0$) with total IgE level.

When comparing the BP with the control group, no significantly elevated levels of IgE-BP Abs (BP180 and 230) in the patients without BP were seen, independent of the total IgE level.

Conclusion: An association between IgE BP antibodies and total IgE-level was found for IgE anti-BP230 Abs with total IgE level >500 kU/l but not for IgE anti-BP180 Abs in BP. Even in the control group patients with total IgE levels >500 kU/l, no significantly elevated IgE BP antibodies were found in comparison to patients with BP.

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Lessons on the immunopathology of epithelial stem cell destruction and antibody-induced cicatricial alopecia from a passive transfer epidermolysis bullosa acquisita (EBA) mouse model

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Irreversible destruction of hair follicle (HF) epithelial stem cells residing in an immunologically privileged niche (the bulge), can cause cicatricial alopecia (i.e. permanent, scarring hair loss). Cicatricial alopecia is also a prominent feature of the mouse model for epidermolysis bullosa acquisita (EBA). Therefore, it offers an excellent experimental tool for investigating the mechanisms by which antibody-dependent immune responses damage and destroy adult epithelial stem cells. For this purpose, skin from the back of C57/Bl6 mice treated for 12 days with rabbit-anti type VII collagen IgG antibodies or with appropriate controls was analysed by quantitative (immuno-) histomorphometry of the bulge region and other defined reference areas. Antibodies to type VII collagen induced a collapse of the bulge immune privilege (e.g. significant up-regulation of MHC class Ia molecules and down-regulation of immunoinhibitory CD200 expression). In addition, they upregulated the number of perifollicular MHC class II+ cells, CD8+, CD4+ T cells and mast cells, but not of neutrophils or gamma/delta TCR+ lymphocytes in and around the bulge of injected mice compared to controls. Furthermore, the immunoreactivity for nerve growth factor (NGF), alpha melanocyte stimulating hormone (αMSH) and interleukin-15 (IL-15), but not for transforming growth factor-beta1 (TGF-β1), was significantly increased in the HF epithelium of test mice. Interestingly, in the bulge regions of EBA mice, the number of proliferating cells (Ki67+) was upregulated. Blocking of the Fcγ IV receptor (with the antibody 9E) in mice of the passive EBA model showed a significantly decreased severity of alopecia, suggesting a protective role of the bulge immune privilege. In these mice staining intensity of MHC class Ia was decreased and reduced numbers of MHC class II+ cells and mast cells were found compared to rabbit anti type VII collagen treated mice.

In conclusion, in addition to their implications for the pathogenesis of EBA-associated cicatricial alopecia, these data suggest that auto antibodies can lead either to epithelial stem cell destruction by a direct attack, or by secondary responses evoked by collapse of the immune privilege. Immune privilege protection and restoration strategies may therefore be well-advised in autoimmune phenomena characterized by irreversible damage to epithelial stem cells.

P149

Gene and protein expression of AIRE (Autoimmune Regulator) in alopecia areata compared to normal human scalp skin

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Autoimmune regulator (AIRE) is predominantly expressed in thymic medullary epithelial cells, where it promotes the expression of tissue-restricted antigens during T cell maturation. AIRE expressing epithelial cells prevent autoimmunity by inducing apoptosis of autoreactive T cells. Mutations in the AIRE gene cause autoimmune-polyendocrinopathy candidadiasis ectodermal dystrophy (APECED) and have been shown to be also involved in skin disorders like vitiligo and alopecia areata (AA).Genetic studies have demonstrated, for example, that two single nucleotide polymorphisms in AIRE are strongly associated with AA patients. The pathogenesis of AA is unknown so far, but a collapse of immune privilege (IP) and an attack of autoreactive T cells against melanogenesis-associated peptides are considered to play an important role. Therefore, the recent discovery of extra-thymic AIRE expressing cells in the lymph node as a second line of defense has prompted us to investigate the presence of AIRE in the relatively immune privileged hair follicle (HF).

By using immunohistology and quantitative histomorphometry on normal healthy human scalp skin and AA specimen, we report, for the first time, the presence of AIRE expressing cells in the immunologically privileged HF. Immunofluorescence demonstrates AIRE expression in the outer root sheath (ORS) keratinocytes, matrix melanocytes of the hair bulb, and in the highly differentiated inner root sheath keratinocytes. These data were additionally supported by mRNA isolation and RT-PCR (AIRE exon 12–13) of isolated HFs. In addition, we show that AIRE protein expression is significantly decreased in the epidermis and HF ORS in AA patients compared with controls.

These observations provide first gene and protein evidence that AIRE is expressed inhuman HFs and suggest that AIRE might play a role in the regulation of tissue-restricted antigen expression in the hair bulb, in the maintenance of HF IP and/or in the development of AA.

P150

Curcumin protects from autoimmune disease by modulating DC and inhibiting Th1/Th17 responses

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Curcumin (diferuloylmethane) is a naturally occurring yellow pigment isolated from the rhizomes of the plant Curcuma Longa. It has been reported that curcumin may possess anti-inflammatory activities and therefore is traditionally used in inflammatory disorders in some regions of Asia. However, the underlying mechanisms that could explain its beneficial activity during inflammation are not fully understood. In this study we analyzed whether curcumin is able to modify immune responses *in vitro* and *in vivo*. First, we investigated the potential anti-inflammatory activities of curcumin on mouse dendritic cells (DC) *in vitro*. Gene expression of curcumin treated DC or control DC activated through TLR4 was analyzed by PCR-Microarrays. Curcumin treated DC showed an upregulation of genes of the TNF super family, Casp1, Casp8and certain NFκB inhibitory genes. Interestingly, curcumin inhibited the production of the inflammatory cytokines interleukin (IL-)12 and IL-23 in TLR4-activated DC. To further investigate the potency of curcumin to modulate immune responses *in vitro* we isolated CD4+ T cells from SJL mouse immunized with PLP peptide in CFA. These CD4+ T cells were activated with APC and PLP peptide in the presence or absence of curcumin. After re-stimulation, curcumin treated cultures showed an inhibition of the Th1 and Th17 cytokines IFN-g and IL-17 whereas an increased expression of the Th2 cytokines IL-4 and IL-10 was observed. We next analyzed the effects of curcumin *in vivo*. SJL mice were fed with curcumin, immunized with PLP in CFA and pertussis toxin and clinical score of experimental autoimmune encephalomyelitis (EAE) was determined. Whereas control mice developed severe EAE, mice treated with curcumin remained healthy or developed only mild disease. The protection from EAE by curcumin treatment was associated with a suppression of IL-12/IL-23p40 and subsequent Th1 and Th17 responses *in vivo*. In contrast curcumin treatment induced IL-4 and IL-10 in CD4+ T cells after active immunization. Even though the molecular mechanism, by which curcumin interacts withIL-12/IL-23p40 expression, is still unclear, curcumin treatment seems to be a promising therapeutic approach for autoimmune disease by using a natural extract.

P151

In vivo measurement of hypoxia-induced angiogenesis using18F-Fluoromisonidazole [(18F)FMISO] as a new powerful tool to detect initial phases of angiogenesis in autoimmune diseases

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Early detection of arthritis is essential to prevent bone destruction by means of interventional anti-inflammatory treatment. Hypoxia can induce angiogenesis via stabilization of the transcription factor hypoxia inducible factor (HIF)-1α and consequently by up regulation of pro-angiogenic mediators. The aim of our study was to examine initial phases of angiogenesis in autoimmune diseases such as rheumatoid arthritis by detection of hypoxia or inflammation, *in vivo*, even before incidence of clinical symptoms or histologically visible joint inflammation. Using positron emission tomography (PET) we investigated hypoxia with the radiotracer [18F] FMISO that selectively accumulates in hypoxic tissue. Moreover we studied glucose metabolism as marker of inflammation, by [18F] Fluordesoxy-glucose [(18F)FDG], *in vivo*. To this end, we induced arthritis in BALB/c mice via intraperitoneal injection of auto-antibodies against glucose-6 phosphate-isomerase(GPI). Mice underwent [18F] FMISO-, or [18F] FDG *in vivo* PET investigations 6–52 h after inflammation induction. Additionally, we performed H&E-staining and real-time PCR analysis of gene expression patterns of joint tissue 6 and 12 h after onset of arthritis. *In vivo* PET images confirmed accumulation of the hypoxiatracer in arthritic ankles shortly after arthritis induction. [18F] FMISO uptake in arthritic joints was significantly higher than in healthy joints even 6 h after GPI serum injection. Importantly, no increase in [18F] FDG-uptake and no visible signs of inflammation were detectible in H&E-stained joint sections 6 h at this time. In line with the PET-data, RT-PCR analysis showed a 7.5 fold enhanced expression of HIF-2αmRNA, 6 h after GPI serum injection, and an up to 12 fold induction of mRNA expression of the pro-inflammatory mediators IL-1β and IL-6. Interestingly, mRNA of pro-angiogenic mediators such as bFGF and VEGF were not yet elevated at this early time point. Thus, non invasive *in vivo* examination of hypoxia-induced angiogenesis using [18F] FMISO is a new, powerful tool to detect initial phases of angiogenesis in autoimmune diseases such as psoriasis arthritis even before joint inflammation becomes detectable by other methods, including the highly sensitive [18F] FDG-PET, used in clinical practice.

P152

Non-invasive *in vivo* imaging of the migration properties of [64Cu] PTSM labelled Th1 cells in mouse models for inflammation

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Non-invasive imaging of T cell trafficking is an important tool for preclinical studies to trace T cell mediated autoimmune diseases and T cell mediated cancer rejection, *in vivo*. In previous studies we focused on intracellular labelling of T cells using the lipophilic radioactive marker [64Cu] PTSM. Investigating viability and functionality of IFN-γ producing ovalbumin-T cell-receptor transgenic T-helper cells (OVA-Th1) revealed that [64Cu] PTSM labelling allows *in vivo* imaging for up to 48 h. The goal of our continued studies was to visualize the migration of OVA-Th1 in an animal model for delayed-type hypersensitivity reaction (DTHR) and of lung inflammation non-invasively *in vivo* over days by positron emission tomography (PET) and computed tomography (CT). To investigate cutaneous DTHR mice were sensitized at the abdomen and one week later DTHR was elicited at the right ear with TNCB; for the induction of OVA-specific lung inflammation, mice were sensitized i.p. with OVA and 4 weeks later challenged intranasally twice. We performed static PET-scans in combination with CT, autoradiography, biodistribution, and FACS analysis 24 and 48 h after OVA-Th1 injection. Analysing T cell trafficking in cutaneous DTHR we detected a 1.5-fold enhanced [64Cu] PTSM-Th1 accumulation selectively in draining cervical lymph nodes of the TNCB-treated right ear compared to the lymph nodes of the untreated left ear. Analysing OVA-specific Th1 migration in lung inflammation we found accumulating [64Cu] OVA-Th1 cells in lung tissue already 24 h after the final challenge. Data were confirmed *ex vivo* by biodistribution, autoradiography, and FACS-analysis 24 h and 48 h after OVA-challenge. These techniques allow for the first time to quantitatively trace specific immune responses with as little as 500 T cells *in vivo*.

P153

Staphylococcus aureus derived monomeric peptidoglycan is a NOD2 ligand and aggravates TLR mediated inflammation by amplifying Th1 and Th17responses

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Staphylococcus aureus causes severe acute infections, life-threatening sepsis, and is a co-factor of chronic inflammatory diseases such as atopic dermatitis (AD). Innate immune sensing of *S. aureus* may cause harmful inflammation. During innate immune sensing cell wall components of *S. aureus* are recognized by pathogen recognition receptors (PRR). The most important *S. aureus* PRR ligands are peptidoglycan (PGN), lipoteichoic acid (LTA), and lipoproteins (LPP). To characterize their exact role and interactions, polymeric PGN was purified from wildtype *S. aureus* and from *S. aureus* deficient in either LPP or LTA. NFkB reporter assays revealed that PGN mediated activation depended on the presence of LPP or LTA, indicating that PGN itself may not activate PRR. For further investigations pure PGN preparations devoid of LPP or LTA activity were needed. Therefore, PGN was enzymatically digested into fragments that are also naturally released from bacteria, purified by HPLC and this monomeric PGN was analyzed for its role in innate immune sensing. In contrast to stimulation with other PRR ligands, dendritic cells(DC) remained immature and produced no cytokines after incubation with monomeric PGN alone. However, monomeric PGN significantly enhanced IL-12p70 and IL-23levels induced by different TLR ligands. To define the coactive pathway utilized by monomeric PGN, DC deficient in NOD2 proteins were analyzed. Strikingly, amplification of IL-12p70 and IL-23 production by monomeric PGN was completely abolished in DC lacking NOD2 identifying monomeric PGN as a natural NOD2-ligand.To investigate consequences on adaptive immune responses DC-T-cell cocultures were set up. Dual activation of DC with TLR and NOD2 ligands lead to enhanced IFN-γ and IL-17 production and diminished IL-4 levels indicative for preferential induction of Th1 and Th17 cells while suppressing Th2 cells.

These data demonstrate that monomeric PGN is an active and potent *S. aureus* PAMP and acts in a newly discovered amplifying circuit of *S. aureus* innate immune sensing. This amplifying circuit finally boosts pro-inflammatory Th1 and Th17responses and suppresses Th2 cells leading to sustained inflammation as also seen in chronic atopic dermatitis.

P154

Injection of antimicrobial peptides as well as disruption of the epidermal barrier affect the adaptive immune response via induction of regulatory T cells

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Ultraviolet radiation (UV) suppresses the adaptive immune response in an antigen-specific fashion via induction of regulatory T cells (Treg). In contrast, the innate immune response appears to be induced by UV, as recently demonstrated for the release of antimicrobial peptides (AMPs), thereby fostering the defense against microbial attacks. We were interested to study whether UV-induced AMPs are involved in UV-mediated immunosuppression. For that purpose we used murine betadefensin (mBD)-14, which is the murine homologue of human BD-3. UV exposure of mice resulted in an increased *in vivo* expression of mBD-14, as demonstrated by immunohistochemistry. C57BL/6 mice were injected intravenously (i.v.) with mBD-14before sensitization with 2,4-dinitrofluorobenzene. 5 days later ear challenge with the same hapten was performed. The ear swelling response was significantly reduced upon injection of mBD-14. Injection of mBD-14 also resulted in the induction of Tregas demonstrated by adoptive transfer experiments. In addition, an increase of the expression of the Treg marker Foxp3 was detected by FACS analysis in T cells obtained from mBD-14 injected mice. Since disruption of the epidermal barrier also induces the expression of mBD-14 and since UV disrupts the epidermal barrier, we asked whether disruption of the epidermal barrier by itself can also suppress the contact hypersensitivity (CHS) response. This was achieved by tape stripping. Application of haptens onto tape stripped skin did not result in sensitization but induced Treg as demonstrated by adoptive transfer experiments. As observed for the injection of mBD-14, T cells obtained from tape stripped mice revealed an increased expression of Foxp3. There is evidence that Langerhans cells damaged by UV are essentially required for the generation of UV-induced Treg. Interestingly, tape stripping increased the number of damaged antigen presenting cells in the draining lymph nodes, implying that barrier disruption might induce Treg in a similar fashion like UV. Disruption of the epidermal barrier supports the penetration of allergens and the induction of CHS. Hence, induction of tolerance via generation of Treg may represent a counter-regulatory mechanism preventing us from excessive sensitizations. We postulate that concurrently induced AMPs might be involved in this potential protective mechanism.

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Identification of TRAIL+ dendritic cells in cutaneous lupuserythematosus

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Background: Cutaneous lupus erythematosus (CLE) is an autoimmune skin disorder characterized by a lesional type I IFN-driven cellular immune response. Earlier analyses provided clear evidence that cytotoxic lymphocytes, which act via granzyme B and perforin, play an important role in the formation of CLE skin lesions. However, the relevance of other cell types, including cytotoxic DCs, remained unclear.

Methods: The expression of TNF-related apoptosis-inducing ligand (TRAIL) on different cell types in CLE skin lesions and on PBMCs was investigated by immunohistochemistry, immunofluorescence and flow cytometry in CLE patients ($n = 10$) and healthy donors ($n = 7$). Lymphocyte culture experiments were performed to investigate TRAIL induction through IFN alpha stimulation.

Results: Our analyses revealed a significant number of TRAIL-positive CD11c+dermal DCs, and also CD123+ plasmacytoid DCs in CLE skin lesions. FACS analyses of PBMCs, taken from CLE patients in stages with active disease, showed a strongly elevated TRAIL expression on CD11c+ cells. Stimulation with IFN alpha *in vitro* clearly enhanced the expression of TRAIL on CD11c+ and BDCA2+ DCs, but also CD3+ and CD56+ lymphocytes.

Discussion: TRAIL-dependent cell death mechanisms appear to be involved in the pathogenesis of CLE. IFN alpha, which is a major lesional proinflammatory cytokinein this disease, enhances TRAIL expression in different PBMC subsets and may be a major stimulus for the lesional 'cytotoxic' DCs which were found.

P156

Generation of monoclonal antibodies against human regulatory T cells to modify their functional activity *in vivo*

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Regulatory T cells (Tregs) control immune homeostasis by suppressing unwanted effector responses. The manipulation of Treg activity therefore represents an exciting possibility to impair pathological immune responses such as in allergy, autoimmunity and cancer. However, efforts to clinically exploit Tregs by interfering with their functional activity require biologicals that affect Tregs selectively. The generation of monoclonal antibodies (mAb) against human Tregs offers the possibility to identify cell specific markers that represent targets for a functional manipulation of these cells *in vivo*. Surprisingly, no report on the generation of specific mAb to Tregs has been published yet.

Here we present a successful strategy to induce and select mAb against human Tregs by repeated immunization of mice with human Tregs isolated from a single leukapheresis combined with a differential flow cytometry-based hybridoma screening procedure. In addition, we describe the antigen identification and functional evaluation for one of the first generated antibodies. The outlined strategy may complement gene-expression studies and provide new tools for the rapeutic modulation of Tregs.

P157

Solid core nanoparticles as carriers in dendritic cell based immunotherapy

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Dendritic cells (DC) are key players in the orchestration of immunological events. To fulfill their role, DC are able to take up antigens via a variety of mechanism, to react to all kinds of endogenous signals, to sense endogenous as well as exogenous danger signals and to integrate all of this into an adequate response. Moreover, DC consists of several subpopulations distinguishable by localization, function and property. To harness the full potential of these cells for immunotherapy, methods to manipulate individual DC subpopulations need to be established. In this project we were examining the application of functionalized, nano-sized, solid core particles (NP) for immunological purposes. NP were phagocytosed by bone marrow derived DC and antigen covalently coupled to the surface was processed and presented on MHC class I and class II to T cells *in vitro*. NP-bound ovalbumin is presented at least100-fold more efficiently than soluble antigen *in vivo*. To specifically activate DC that had taken up NP-bound antigen, CpG was covalently linked. As shown by upregulation of costimulatory markers and release of inflammatory cytokines by DC *in vitro* and priming of CTL *in vivo* CpG-coated NP proved to provide potent activating signals to DC. To improve uptake of particles as well as specific targeting NP were additionally coupled with an antibody against DEC205, a C-type lectin receptor specifically expressed on some DC-subpopulations. *In vitro* experiments show that phagocytosis of NP by DC was strongly enhanced by coupling of aDEC205.Concomitantly, presentation of antigen and activation by CpG coupled to these NP was more efficient. In summary, our results show that the utilization of NP may be away to improve current vaccination strategies.

P158

Decreased expression of RANKL in skin lesions of patients with cutaneous lupus erythematosus

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In the past years, naturally occurring CD4+CD25+ regulatory T cells (Treg) have emerged as a major factor in our understanding of self-tolerance and mechanisms in autoimmune diseases. Moreover, epidermal RANKL has been shown to play an important role in the maintenance of peripheral Treg. Recently, a decrease ofCD4+FoxP3+ Treg has been observed at the site of inflammation in skin lesions of patients with cutaneous lupus erythematosus (CLE) in contrast to psoriasis and lichen planus. To investigate the expression of RANKL in the epidermis of skin lesions from patients with different subtypes of CLE, biopsy specimens from 119patients were analyzed by immunohistochemistry and compared to 25 patients with psoriasis and 18 patients with lichen planus using the tissue microarray (TMA) technique. For evaluation of epidermal RANKL expression in skin biopsy specimens, the relative proportion of positive keratinocytes (level of positivity: 0 = 0%, 1 = up to 1%, 2 = 2–10%, 3 = 11–50%, 4 => 50%) was multiplicated with the value for staining intensity (level of intensity: 0 = negative, 1 = low, 2 = medium, 3 = strong). Only 12 (10.1%) skin biopsy specimens from patients with CLE revealed expression of RANKL in the epidermis compared to 7 (38.9%) from patients with lichen planus and19 (76.0%) from patients with psoriasis. Therefore, RANKL was significantly higher expressed in skin lesions of patients with psoriasis (median: 4, range: 0–5) compared to patients with CLE ($P < 0.001$) and lichen planus ($P < 0.05$) as well as compared to normal healthy donors ($P < 0.05$). However, RANKL expression in skin lesions of patients with CLE (median: 0, range: 0–4) or lichen planus (median: 0, range: 0–4) differed not significantly from normal healthy donors (median: 0, range: 0–0). There were also no significant differences in epidermal RANKL expression between the various subtypes of CLE, but disease duration correlated negatively with the number of patients expressing RANKL in skin lesions. A significant difference was found analyzing RANKL expression in skin biopsy specimens with regard to the presence of antinuclear antibodies in the sera of patients with CLE. Together, these data suggest that RANKL might be important for the development of skin lesions in CLE and possibly provides a therapeutic alternative in future studies.

P159 (V26)

The activating transcription factor 3 (ATF3) determines postseptic immune suppression

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Following severe systemic infections, such as cellulites, fasciitis or measels, patients are at high risk of severe secondary infections of bacterial, mycotic or viral origin. This phenomenon is defined as postinfectious immune paralysis; however the molecular mechanisms behind this phenomenon are elusive. Here we first show a close correlation of a severe decrease of glutathione-levels that significantly correlates with the induction of the activating transcription factor (ATF3) in postseptic patients. We therefore speculated that ATF3 may contribute to postseptic immune suppression and the increased susceptibility to opportunistic infections. ATF3 is a transcription factor in the NF-κB signaling pathway and negatively regulates the transcription of IL6 and TNF. To test this hypothesis we used CLP (cecal ligation and puncture), one of the best-established models of bacterial sepsis. We first induced sublethal CLP in wt and ATF3–/– mice, to closely imitate the clinical conditions. Subsequently we challenged the mice during the postseptic immune suppressive phase with the fungal pathogen *Aspergillus fumigatus*, at doses that are nonpathogenic to healthy mice. Postseptic wt-mice rapidly succumbed to this sublethal pulmonary *Aspergillus fumigatus* infection. In sharp contrast, ATF3–/– mice had not only a significantly prolonged survival, 20% of these mice even survived the lung infection.

Thus, ATF3 is an important regulator of postseptic immune suppression, as it critically determines susceptibility to and the course of opportunistic infections. The data uncover for the first time a molecular mechanism, underlining postseptic immune suppression. By showing, that this immune suppression directly results from glutathione-mediated ATF3 induction, we provide the basis for a rational treatment that counteracts this dangerous immune suppression.

P160

Glucocorticosteroid-treated immature Langerhans cells induce regulatory T cells *in vivo* and *in vitro*

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Although glucocorticosteroids have been used for many decades in auto immune diseases and transplantation, the exact mechanisms responsible for their immunosuppressive properties are not fully understood.

We therefore undertook a study with 24 nickel-allergic patients, performed epicutaneous patch tests (EPT) prior and after oral steroid or placebo treatment and took skin biopsies 72 h after EPT application for immunofluorescence and quantitative RT-PCR analysis. In addition we performed functional experiments with cord blood-derived Langerhans cells (LC), co-cultured them with T cells and analyzed the quantity and quality of regulatory T cell induction.

Expectedly, oral steroid treatment led to a substantial reduction of clinical symptoms that was paralleled by a sizeable decrease of infiltrating T cells. However, we found an increase of FoxP3+CD25+CD4+ T cells in the dermis of post-steroid EPT as compared to the placebo control group. In addition, higher numbers of epidermal LC were observed after oral steroid treatment in comparison to the placebo group. In contrast to LC from pre-steroid patch tests, those after steroid had an immature phenotype. Interestingly, increased numbers of epidermal LC and dermal regulatory T cells were associated with upregulated expression of TGF-beta in skin biopsies after steroid treatment. The relation between immature LCs and FoxP3+ regulatory T cells was further underscored by functional experiments that showed an induction ofFoxP3+CD4+ T cells with suppressive activity when T cells were incubated with dexamethasone-pretreated LC, which was not the case in the absence of dexamethasone or after coincubation of T cells and myeloid or plasmacytoid dendritic cells.

Our data support the concept that corticosteroid can promote the generation of regulatory T cells and, thus, shed new light on the mechanisms of glucocorticosteroid-mediated immunosuppression.

P161

Anti-annexin 1 antibodies: a new diagnostic marker in the serum of patients with discoid lupus erythematosus

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Apoptotic cells appear to be a crucial source of antigenic targets of systemic autoimmunity, and there are several reports describing clearance deficiency as a possible mechanism in the pathogenesis of systemic lupus erythematosus (SLE).Several defects have been described wherein the disposal of apoptotic cells is compromised, thus leading to their accumulation in parenchymal organs. Moreover, this can result in the generation of autoantibodies, such as anti-Sm antibodies, which are characteristically present in patients with SLE and are thus used for diagnostic purposes. One common target of autoantibodies is the intracellular molecule, annexin1, which is externalized during apoptosis and binds to phosphatidylserine on the outer leaflet of the plasma membrane. The aim of the present study was to determine whether antibodies against annexin 1 can be detected in the serum of patients with cutaneous lupus erythematosus (CLE) and to assess whether they are a useful diagnostic tool for this disease. We studied sera from 78 patients with different subtypes of CLE, such as subacute cutaneous lupus erythematosus, discoid lupus erythematosus (DLE), and lupus erythematosus tumidus. As controls, we analyzed sera from 26 patients with other connective tissue diseases (CTD) and from 56 normal healthy donors (NHD). Detection of anti-annexin 1 antibodies was carried out by a new established ELISA. Moreover, we correlated different clinical features of CLE patients with their level of anti-annexin 1 antibodies. Anti-annexin 1 antibodies were detected at significantly higher levels in the sera of patients with CLE and other CTD as compared to NHD ($P < 0.001$). Additionally, the percentage of sera positive for anti-annexin 1 antibodies was elevated in patients with CLE as compared to NHD. In particular, the percentage of sera positive for anti-annexin 1 antibodies was significantly higher in patients with DLE as compared to NHD ($P < 0.001$). Some clinical and serological parameters, such as photosensitivity, showed a positive correlation with anti-annexin 1 antibodies; however, disease activity and treatment with systemic corticosteroids did not influence the levels of anti-annexin 1 antibodies. The results of this study indicate that anti-annexin 1 antibodies in the sera of patients with DLE might be a valuable aid in the identification of this CLE subtype.

P162

Unique expression, regulation and function of Fc gamma RIII (CD16) on human lan-dendritic cells

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The differential expression of Fc receptors provides dendritic cell (DC) subtypes with a specific functional repertoire. We previously described 6-sulfo-LacNAc expressing DCs (slanDCs) in human blood which stand out by their high level expression of the low affinity Fc gamma receptor III (CD16). SlanDCs are highly proinflammatory, serve as the major and early source of IL-12, IL-1 beta and TNF-alpha and can become inflammatory dermal dendritic cells in psoriasis and lupus erythematosus. During maturation expression of CD16 is rapidly lost while maturation markers such asCD86, CD80, CD83 and MHC class II are upregulated. Here we describe the slanDC-specific function of CD16 and its sheddase-mediated release. Comparative binding studies of IgG complexes to slanDCs, CD1c+ DCs, pDCs and monocytes revealed that the selective expression of CD16 specifically enabled slanDCs to bind IgG immune complexes with a much higher capacity compared to the other cell types studied. CD16 but not CD32 also proved critical for slanDCs to phagocytose IgG-opsonized sheep red blood cells. Finally, a higher proliferation of antigen-specific T cells was observed when antigen was targeted to CD16 instead of CD32 on slanDCs. The rapid downregulation of these functions during slanDC maturation is concurrent to a loss of CD16 expression. This loss of CD16 expression could be inhibited by the presence of non selective metalloproteinase inhibitors (1,10-phenanthroline, EDTA, GM6001). Using a complementary set of selective metalloproteinase inhibitors (Ro32-7315, Ro32-7066, Ro4002855, Ro28-2653, GW280264x, GI254023x) we identified ADAM17 and ADAM10 but not matrix metalloproteinases (MMPs) as responsible for cleaving CD16 on slanDCs. Taken together, the expression of CD16 equips slanDCs with a unique capacity to capture antigen for T cell presentation that is restricted to their immature stage and which is controlled by the action of ADAM17 and ADAM10. Given the fact that slanDCs can become inflammatory dermal DCs, the high capacity to capture immune complexes adds an important function to these cells in terms of bridging innate and adaptive immunity.

P163

The functional spectrum of TLR7/8-triggered DC cytotoxicity

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Dendritic cells (DCs) do not only exhibit the unique capacity to evoke primary immune responses by presenting antigens to naïve T cells, but may also acquire Toll-like receptor (TLR)-triggered cytotoxic molecules. While TLR7/8- andTLR9-stimulated plasmacytoid DCs (pDCs) isolated from human peripheral blood express tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), TLR7/8-stimulated myeloid DCs (mDCs) express perforin and granzyme B, but not TRAIL. We now wanted to gain a better understanding of the molecular players involved in DC cytotoxicity. Using a range of TLR 1–9 agonists we show that the induction of cytotoxic molecules is subtype-specific and only inducible with intracellular, but not extracellular TLR agonists. In both pDCs and mDCs cytotoxic molecule expression was accompanied by phenotypic maturation and did not prohibit the induction of an intact immune response as determined in an MLR. In the case of pDCs, but not mDCs, TLR7/8-induced killer molecule expression was IFN-alpha-dependent and, consistently, TRAIL-expression on pDCs could be induced by IFN-alpha stimulation. At a functional level both TLR7/8- and IFN-alpha-stimulated pDCs killed Jurkat T cells in a TRAIL-, IFN-alpha- and cell contact-dependent fashion. In contrast, TLR7/8-stimulated mDCs lysed the MHC I low tumor cell line K562 in a perforin-dependent fashion, but much less efficiently their HLA-A-transfected counterpart. Since natural killer (NK) cells are able to kill HLA-A-transfected K562 cells and mDCs lack the phenotypic profile of NK cells, these findings indicate that, although they use the same killing mode, NK cells and mDCs recognize different targets.

In conclusion our data demonstrate two distinct mechanisms by which pDCs and mDCs elicit their tumoricidal activity, pointing to an as yet underappreciated powerful innate defense line in infectious and tumor immunity.

P164 (V36)

Impaired dectin-1 signalling in patients with chronic mucocutaneouscandidiasis

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Chronic mucocutaneous candidiasis (CMC) constitutes a selective inability to clear infection with the yeast Candida albicans resulting in persistent debilitating inflammation of skin, nails, and mucous membranes. To date the underlying defect is unknown. In order to characterize cellular immunity in patients with CMC including patients with autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED), we analysed the response of dendritic cells (DCs) to Candida albicans. Furthermore, differentiation to and effector-functions of Th17 cells – recently described to be involved in the clearance of candida infections – were analysed. PBMCs of 13 CMC patients (7 with APECED) and 12 healthy controls were stimulated with candida, PHA and aCD3/CD28 for 72 h. DCs of both groups were incubated with the candida cell wall compounds zymosan and curdlan and LPS as control. T cell proliferation capacity was investigated by thymidine incorporation; cytokine production both quantified in supernatants by ELISA or intracellularly by flow cytometry. Candida-specific IFN-γ production was reduced in CMC patient, while IL-10 production was markedly higher. This effect was independent from the proliferation capacity of PBMC as this effector function was comparable in CMC patients and healthy controls. Importantly, T cells from CMC patients secreted significantly lower amounts of Th17-associated cytokines IL-17A and IL-22. The release of the cytokines IL-10 and TNF-α that are specific for dectin-1 signalling is abrogated in curdlan-matured DCs of CMC patients compared to healthy controls. Treatment of DCs with curdlan also resulted in decreased levels of Th17differentiating cytokines IL-6, IL-1β and IL-23 in CMC patients. Our data indicate that the inability to clear the yeast Candida albicans in CMC patients seems to be due both to an altered innate and an altered adaptive immune response to dectin-1mediated signalling. A whole genome analysis of DCs and T cells after stimulation with curdlan of both groups is currently under investigation. The impaired pathogen recognition through a dysregulated dectin-1 signalling after stimulation with curdlan and the following impaired antimicrobial response could play a central role in the pathogenesis of chronic mucocutaneous candidiasis.

P165

Clinical significance and regulation NKG2D ligands in melanoma

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The immunoreceptor NKG2D acts as a main stimulatory receptor on NK cells and has co-stimulatory function on different subsets of T cells. As several mouse studies underline the importance of NKG2D in tumor immunosurveillance we analysed cultured human melanoma cells for the surface expression of the NKG2D ligands(NKG2DL) MICA, MICB, ULBP1, ULBP2 and ULBP3. Of these five ligands we found MICA and ULBP2 predominantly expressed *in vitro*, suggesting that NKG2D might be involved also in immunosurveillance of melanoma. However, tumors are known to escape from NKG2D recognition by shedding its ligands, rendering the soluble products detectable in patients' sera.

To elucidate the clinical significance of MICA and ULBP2 in melanoma, we subsequently studied their expression on tumor tissues and their presence as soluble molecules in sera from more than 200 patients and compared the latter to the well-established serum marker S100B. Immunohistochemical analysis on melanoma metastases revealed a very heterogenous expression of MIC and ULBP2 between and even within tumors. Compared to MIC, ULBP2 was less frequently expressed. Accordingly, elevated levels of soluble (s) ULBP2 were detected in sera of melanoma patients less frequently than elevated levels of sMICA. Strikingly, only elevated concentrations of sULBP2 were strongly associated with disease progression and tumor load. Elevated serum levels of either sNKG2DL correlated with reduced overall survival, albeit considerably stronger for sULBP2 ($P < 0.0001$) than for sMICA ($P = 0.011$). In early-stage (I–III) melanoma patients only sULBP2 ($P < 0.0001$), but neither sMICA nor S100B revealed prognostic significance. Multivariate analysis identified sULBP2 ($P = 0.0015$) and S100B ($P = 0.013$), but not sMICA as independent predictors of prognosis. Our data indicate that 1) expression of single NKG2D ligands in melanoma is regulated differently and that 2) particularly sULBP2 is of clinical relevance as a strong indicator of poor prognosis. These observations prompted us to study the regulation of NKG2DL expression in melanoma. On going studies suggest that both MICA and ULBP2 are under control of aberrantly activated oncogenic signalling pathways but via different mechanisms.

P166

Regulation of IgE-production *in vitro* by $CD8^+CD28^+$Perforin-containing T regulatory cells via Suppression of IL13 in Patients with Atopic Dermatitis

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Recently, a role of $CD8^+$ T cells in human IgE-control *in vitro* was demonstrated. Cytokines and the $CD8^+$ subpopulation involved remained unclear. Therefore, ficoll-isolated peripheral mononuclear cells (PBMC) were obtained from 15 patients with acute exacerbated extrinsic atopic dermatitis (medium to high SCORAD, serum IgE levels 500–8000 U/ml). Levels of interleukin-4(IL4), IL12, IL13 and interferon-gamma (INFg) were determined in the culture supernatant over a time period of 10 days by immunoflow cytometry ($n = 8$, FCAP Array, Becton Dickinson) of (i) PBMC, (ii) PBMC depleted of $CD8^+$ T cells by Milteny beads and (iii) CD8-depleted PBMC reconstituted with$CD8^+$ T cells. In addition, total *in vitro* IgE-levels were measured (Pharmacia UniCAP system). In a separate set of experiments ($n = 7$), additional experimental conditions were included: CD8-depleted PBMC reconstituted with (iv) $CD8^+$ $CD28^-$ or (v) $CD8^+CD28^+$ T cells. Depleting >90% of $CD8^+$ T cells *in vitro* elevated IgE-levels significantly as compared to PBMC. Normalization of IgE-production after reconstitution with $CD8^+$ T cells was mediated exclusively by the $CD28^-$ subpopulation. IL13- and INFg-levels rose significantly after removal of $CD8^+$ T cells and returned to control levels after reconstitution. IL13-levels correlated with IgE-levels *in vitro*.IL4/IL12-levels were not altered by CD8-depletion. $CD8^+CD28^-$ T cells belong to the family of regulatory T lymphocytes. They contain the majority of perforin-granules which were shown to be involved in IgE-control in mice and men. Our data now suggest that $CD8^+CD28^-$ perforin$^+$ T regulatory cells control IgE-production *in vitro* by regulation of IL13-production.

P167

Dendritic cells loaded with HSP70-expressing heat-killed melanoma cells efficiently activate autologous T cells

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There is considerable interest to develop strategies that enhance cross-presentation pathways of dendritic cells (DCs) to elicit strong cytotoxic T cell responses in cancer vaccination therapy.

In order to best exploit the enhanced cross-presentation of antigens bound to heat shock protein 70 (HSP70), we analyzed melanoma cell preparations for their HSP70expression. For generation of heat-necrotic cell material melanoma cells were kept at42°C for 90 minutes and then heated to 56°C for a final 30 minutes. Apoptotic Annexin Vpos. /PI neg. melanoma cells were generated by irradiation with 1.0 J/cm^2 UV-B. Western blotting revealed strong upregulation of HSP70 after heat-killing in contrast to UV-B irradiation. When the uptake of heat-killed necrotic cells by DCs at various levels of maturation was assessed, 61% ± 7% of immature DCs (iDCs) internalized fluorescence-labeled necrotic material. Apoptotic material from UV-B-irradiated cells was internalized by only 48% ± 5% of iDCs. Maturation-inducing cytokines did not affect the uptake when added simultaneously with the tumor cell preparations. Loading DCs with heat-necrotic or apoptotic melanoma cells slightly reduced CD83expression while leaving CD208 (DC-LAMP) expression unchanged. As determined by IFN-γ-detecting ELISPOT assays, iDCs loaded with heat-killed melanoma cells activated autologous T cells most effectively when used without any further maturation, whereas DCs loaded with apoptotic material required maturation. In conclusion, HSP70-expressing melanoma cells could be generated by heat-killing. Loading immature DCs with heat-killed melanoma cells resulted in a superior priming of autologous T cells *in vitro*.

P168 (V33)

Analysis of the effect of IL-10-modulated DC in a humanized mouse model of lethal GvHD

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In non-inflammatory conditions, the most prominent task of the immune system is to keep a tolerant state to innocuous agents and self antigens. To understand the mechanisms of tolerance, various systems have been analyzed *in vitro*, in part involving immature and tolerogenic dendritic cells. We have previously shown *in vitro* that IL-10 modulated human dendritic cells (IL10DC) display a tolerogenic phenotype, associated with low expression of MHC II and costimulatory molecules and impaired production of proinflammatory cytokines. IL-10DC induce energy in CD4+ and CD8+T cells. These T cells exhibit suppressor effects on the function of effector T cells *in vitro* and are, thus, characterized as induced regulatory T cells (iTreg). iTregs might be exploited therapeutically for suppression of cellular immune responses in severe allergic and autoimmune diseases or transplantation rejections (e.g. graft versus host disease, GvHD). As a preclinical screening system for the *in vivo* efficacy of tolerogenic IL-10DC, we choose a model of GvHD in humanized NOD-Scid mice. Transfer of human PBMC into newborn NOD-Scid mice resulted in lethal GvHD within 2–3 months, characterized by growth retardation, weight loss, inflammatory reaction of the skin and multiple organs and death of affected animals. As a first approach, IL-10DC were used which were not additionally loaded with murine antigen. Treatment of GvHD with a single (at time point of PBMC transfer) or multiple doses (3× in weekly intervals) of unloaded IL-10DC, did neither prevent growth retardation, nor affect symptom-free time and overall survival of the animals to a significant extent. Human IL-10DC, which express CXCR4 but reduced levels of CCR7, did reach secondary lymphoid tissue as demonstrated by recovery from spleen and lymph nodes. Organ infiltration (lung, skin) by human cells and the percentage of human CD4+ in spleen, lymph nodes and peritoneum were comparable in animals treated with IL-10DC and controls. The percentage of CD8+ T cells of animals treated one time with tolerogenic DC showed no difference to controls whereas multiple treatment result in a decrease of infiltrating CD8+ T cells. These data show, that *in vitro* generated human IL-10DC without additional antigen loading are able to reach secondary lymphatic tissues in NOD-SCID mice but do not modulate experimental lethal GvHD to a significant extent.

P169

Analysis of patients under long term treatment with fumaric acid esters for upto 4 years

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Psoriasis is one of the most common chronic inflammatory skin diseases and affects about 2–3% of the Caucasian population. This corresponds to about 1.6 Mio. People in Germany alone. Approximately 20% of these patients need systemic treatment due to the severity of the disease. The most frequent used systemic first-line treatment of psoriasis in Germany is the oral application of a fixed combination of fumaric acid esters (Fumaderm®). It is preferably used when long term treatment is required. Surprisingly, there are no long term data from large prospective, randomized clinical trials about efficacy and safety of this treatment. This let us retrospectively analyze the data of 47 patients receiving treatment with fumaric acid esters for a time period of up to 4 years. Besides the medical history of the patients and the data concerning the severity of psoriasis (clinical phenotype, severity of the skin lesions, affection of the nails and joints) we collected data about concomitant diseases and adverse events that occurred during the treatment. We also collected laboratory parameters like blood cell counts, transaminases, creatinine and blood lipid levels as well as urine samples. Furthermore, we investigated numerous immunologic parameters like number of CD4+ T cells. We found that in the beginning of the treatment the most frequent adverse events were gastrointestinal complaints and this corresponds well to the known side effects of the treatment. Interestingly we observed that after longer treatment periods there was a clear increase of specific infections. More importantly, it appears that these infections occurred in relation to particular preceding immunological changes. We expect that the control of selected immunological parameters may permit a safer long term treatment with fumaric acid esters for the patients.

P170

Uptake of protein antigen into various subsets of skin dendritic cells

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Skin dendritic cells (DC) acquire exogenous antigens by several different mechanisms and the help of different surface receptors. Ovalbumin (OVA) is a widely used model antigen. Its uptake into DC has not been studied in detail. We therefore performed experiments using OVA-Alexa conjugates to investigate incorporation and localisation in DC. DC were derived from skin explant cultures or freshly prepared by enzymatic digestion of skin. With immunofluorescence microscopy and Flow Cytometry technique we were capable to visualize and measure the uptake of antigen by DC subsets. After intradermal injection of OVA the epidermal Langerhans cells (LC) showed incorporation of antigen preferentially into endosomal compartments. Furthermore this 'antigen-loading' could be accomplished *in vivo* by topical application of the antigen onto the skin of mice. For this *in vivo* uptake by LC, the disruption of the skin barrier and the induction of an inflammation seemed to be necessary. Irrespective of the present discussion about the *in vivo* functions of LC, these data underscore that LC can efficiently take up protein antigens delivered intradermally or epicutaneously. Moreover this work underlines the importance of LC in skin immunization strategies.

P171

Continuous systemic therapy ameliorates biomarkers of cardio-vascular risk inpatients with severe plaque-type psoriasis: results of a prospective longitudinal observational study

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Psoriasis patients exhibit an increased cardiovascular mortality. In order to investigate the effects of continuous systemic therapy on the cardiovascular risk of patients with severe plaque-type psoriasis, we performed a monocentric, prospective longitudinal observational study. 42 consecutive patients with severe plaque-type psoriasis, referred from primary care centers for initiation of systemic therapy, were included. All patients received continuous systemic treatment for their psoriasis according to the German treatment guideline. The clinical course was monitored over 24 weeks. Initially as well as after 12 and 24 weeks, oral glucose tolerance tests were performed along with a comprehensive laboratory monitoring. Main outcome measures of the study were correlations of psoriasis severity, as reflected by the Psoriasis Area and Severity Index (PASI), with biomarkers for cardiovascular risk (e.g. high-sensitive CRP (hsCRP) and indicators of insulin resistance. We found that patients responding to therapy, defined as a reduction in the PASI by at least 50%,showed statistically significant correlations between the PASI and hsCRP ($r = 0.38$, $P = 0.02$) as well as vascular growth factor ($r = 0.46$, $P = 0.005$). Among the adipokines, resistin was positively, and the potentially cardio-protective adiponectin negatively correlated with the PASI ($r = 0.46$, $P = 0.004$ and $r = -0.33$, $P = 0.05$, respectively). Oral glucose tolerance tests yielded positive correlations between the PASI and serum levels for glucose ($r = 0.47$, $P = 0.05$), insulin ($r = 0.63$, $P = 0.005$), and C-peptide ($r = 0.65$, $P = 0.003$) at $t = 120$ minutes in those patients with a pathological HOMA (>2.5), indicating that the state of peripheral insulin resistance is driven at least in part by the severity of the psoriatic inflammation. This is the first prospective study documenting anamelioration of biomarkers of cardiovascular risk in patients with severe plaque-type psoriasis through continuous systemic therapy. These observations challenge the current treatment paradigm of psoriasis. Future studies need to compare the cardio protective effects of different treatment modalities, based on hard clinical endpoints such as the rate of myocardial infarction and death.

P172

Evaluation of the antifungal effect of polyethylenimine (PEI) by laser nephelometry

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Objective: Laser nephelometry measures light scattering by particles suspended in solution at a right or forward angle to the beam. The technique enables the monitoring of microbial growth curves by recording the turbidity of the solution and provides a high sensitivity that allows the detection of even low concentrations of scattering particles which is the case during the lag phase and the beginning of the log phase of microbial growth. Laser nephelometry was employed to evaluate the effect of polyethylenimines on Candida albicans. The yeast is facultative pathogenic and under normal circumstances resides in 80% of the population without harmful effects. However, systemic fungal infections have emerged in immune-compromised patients and hospital-acquired infections have increased. Polycationic substances such as polyhexanid or chitosan have been reported to possess a high antimicrobial activity due to interaction with the microbial membrane structures. Hence, we have tested five different polyethylenimines with a high positive charge density for antifungal activity against Candida albicans using laser nephelometry.

Method: *C. albicans* was precultured in Sabouraud-Glucose medium and a cell count of 8×103 cfu/ml was adjusted. Two linear polyethylenimines with a molecular weight of 2.5 kDa (PEI2.5) and 25 kDa (PEI25), and three branched polyethylenimines with a molecular weight of 5 kDa (Lupasol G100), 25 kDa (Lupasol WF), and 750 kDa (Lupasol P) were tested. According to protocol 100 μl of the test samples were suspended in 96-well plates and 100 μl inoculum was added. Plates were sealed with transparent plastic films punctured with a 14-gauge needle to allow gas exchange. The growth curves were recorded by monitoring the turbidity of the cultures using the NEPHELO star Galaxy (BMG Labtech, Germany).

Results: All polyethylenimines tested significantly inhibited yeast growth. IC50 values were calculated after 24 h: PEI2.5 – 55.9 ± 6.6 μg/ml; PEI25 – 51.0 ± 2.5 μg/ml; LupasolG100 – 15.6 ± 2.7 μg/ml; Lupasol WF – 25.8 ± 1.6 μg/ml; and Lupasol P -19.2 ± 4.6 μg/ml. A statistic significant difference was observed between IC50 values of linear and branched polyethylenimine.

Conclusions: Poly-cationic substances have been shown to possess antifungal properties against yeasts, moulds and dermatophytes. We have evaluated the effect of different polyethylenimines with a high positive charge against Candida albicans using laser nephelometry, a highly sensitive technique for monitoring microbial growth allowing a high through-put screening of candidate substances for antifungal activity. All polyethylenimines tested were able to significantly inhibit yeast growth recommending themselves as promising future treatment possibilities in candidoses and other fungal infections.

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A polyacrylate-superabsorber inhibits the formation of ROS/RNS *in vitro*

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Introduction: The exudates of chronic wounds contain elevated levels of reactive oxygen and nitrogen species (ROS/RNS). This overproduction of free radicals perpetuates the inflammatory phase and results in severe tissue damage. Therefore, the reduction of these active speates while keeping the wound environment moist; an additional binding of matrix degrading proteases by the super absorbers has been shown. A further anti-oxidative effect would be another beneficial attribute of polyacrylate-superabsorbers.

Materials & Methods: Antioxidant potential of a polyacrylate-superabsorber containing wound dressing (Vliwasorb, Lohmann & Rauscher) was measured using the chemiluminescent ABEL® Antioxidant test kits containing Pholasin® specific for superoxide and peroxynitrite (Knight Scientific Limited, UK).

Results: The polyacrylate-superabsorber exhibited a significant concentration dependent antioxidant potential. The wound dressing samples were equally effective in inhibiting the formation of ROS and RNS.

Conclusions: It is believed, that the overproduction of reactive nitrogen and oxygen species in chronic wounds results in an elongated inflammatory phase and severe tissue damage. Hence, the reduction of these active species seems to be a suitable way to promote normal wound-healing. Polyacrylate-superabsorber inhibits the formation of free radicals *in vitro*. Therefore, wound dressings containing Polyacrylate-superabsorbers should have an auxiliary influence on the healing of chronic wounds besides the binding of exudates.

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Clinical stabilization of a rapidly progressing bone metastasizing squamous cell carcinoma using the receptor tyrosine kinase inhibitor erlotinib (Tarceva®)

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Squamous cell carcinomas (SCC) are among the most common cutaneous cancers. Majority of these tumours can be cured in early stages but upon metastases to internal organs the therapy is challenging. Receptor tyrosine kinases (RTK) s are the high affinity cell surface receptors for many polypeptide growth factors, cytokines and hormones. In particular, the RTK EGFR (epidermal growth factor receptor) has been shown to be one of the key regulators in the development and progression of epithelial cancers including SCCs.

Here we present a 70-year old male patient who was admitted to our ward because of chronic pain in right leg and spinal column. Magnetic resonance imaging of the skull, thorax and abdomen showed multiple osteolytic lesions in cervical, thoracic, lumbar and sacral spines as well as a fracture in the right iliac bone suspect of multiple bone metastases. Metastasis at other visceral sites was not detected. The primary care physician reported these lesions to be rapidly progressing within 1 year under a palliative therapy with bisphosphonate and radioactive samarium (as a pain therapy). A biopsy of right iliac bone demonstrated a SCC metastasis, derived presumably from a basosquamous carcinoma (BSCC) with histopathological features of both basal cell (BCC) and SCC at the right temporal region that had had been treated with radiotherapy a year before.

The patient's karnofsky-index was reduced to 50% corresponding to ECOG 2(Eastern Cooperative Oncology Group) score.

A multidisciplinary team of dermato-oncologists, oncologists, orthopaedics, radiologists, physiotherapists and pain specialists got involved. Because of his reduced karnofsky-index and age, chemotherapy was avoided. Immunohistopathology of the SCC metastasis showed abundant expression of EGFR. We started an additional therapy with RTK inhibitor erlotinib (Tarceva®) at the dose of 100 mg qd.

The patient developed a follicular-pustular rash on his back 4 weeks after the start of the therapy. This adverse event has been well established to correlate with the clinical response to RTK inhibitors. The patient developed stable disease which is now ongoing for 15 months under erlotinib therapy. His karnofsky-index improved significantly to 80% and the therapy was accompanied by a significant increase in quality of life.

Finally, we conclude that RTK inhibitors can be considered in patients with metastasizing SCCs which are otherwise not eligible for other conventional therapies.

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German ItchyQoL: a novel instrument to assess pruritus-specific quality of life

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Pruritus is a widely spread symptom in numerous dermatologic and systemic diseases that may have an enormous impact on patients' daily life. The high individual burden of chronic pruritus along with the difficulty in objectively measuring pruritus intensity makes the assessment of quality of life (QoL) a suitable instrument to evaluate the impact of itch upon a patient and the efficacy of its therapy. To date, a QoL instrument that addresses the particular aspects most relevant for pruritus patients which can be applied in pruritus independent of the underlying cause is lacking in German-speaking countries. Recently, the first existing pruritus-specificQoL questionnaire (ItchyQoL) has been created by Desai et al. and proven to be reliable, valid and responsive. In order to develop a German version of the instrument, we translated, culturally adapted and retranslated the original instrument in cooperation with the original authors and performed pilot testing. This resulted in the creation of the first German tool for the assessment of QoL in patients suffering from pruritus. Clinical validation of the questionnaire in a large population of patients with pruritus of any aetiology is ongoing and will enable us to explore the impact of pruritus on QoL. To date, 271 pruritus patients with different underlying diagnoses(79× urticaria, 56× atopic dermatitis, 34× psoriasis, 24× prurigo nodularis, 20× kidney disease, 13× liver disease, 11× malignancies, 34× others/unknown origin) have completed the questionnaire. Preliminary data show that women are affected more often by pruritus than men (ratio 1.8:1) but there are no major differences in mean itch severity between sexes (females 5.3, males 4.8 on a visual analogue scale [VAS] ranging from 0–10) or diseases (ranging from VAS 4.8 for urticaria to VAS 6.2 for liver disease). Future use of this patient-related outcome measure will improve efficacy of treatment in pruritus patients and generate direct patient benefit.

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MCV status does not impact on histological and clinical characteristics of european and australian merkel cell carcinomas

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Since the discovery of a newly identified polyoma virus (MCV) associated with Merkel cell carcinoma (MCC) this tumor experienced much attention. Indeed, several reports meanwhile confirmed the presence of this virus in most of the European and American MCCs. In contrast, a recent report demonstrated a much lower frequency for Australian MCC patients. Moreover, it is still unclear whether MCV status impact son characteristics or clinical outcome. Consequently, we determined the presence of MCV in 165 MCC patients of which 38 were from Australia, and compared the characteristics and overall survival based on MCV status. To this end, we detected MCV DNA in about 85% of MCC patients irrespective of their origin by real time PCR. Notably, immunohistochemistry revealed large T antigen expression also on protein level. Importantly, MCV positive and negative patients displayed similar recurrence-free, overall and MCC-specific survival. In addition, no differences were observed for gender, histological features, age at and year of diagnosis. Furthermore, expression pattern for p16, p53, RB1 and Ki67 were similar. Indeed, the only detectable significant difference was that MCV positive primary tumors were less often located on the trunk and more often on the extremities. Thus, although MCV positive and negative MCC may have different etiologies, they seem not to differ in tumor characteristics or clinical behavior.

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The clinical activity of pemphigus vulgaris is directly linked to IgE autoantibodies reactive with desmoglein3

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Pemphigus vulgaris (PV) represents a severe, potentially life-threatening autoimmune disease characterized by extensive blisters and erosions of the mucous membranes and the skin. A cantholysis of epidermal keratinocytes is caused by autoantibodies against the major autoantigen, desmoglein 3. IgG1 and IgG4 are the commonly found subtypes of autoantibodies, whereas IgG4 is considered to be pathogenic and associated with active disease stage. Since it has been previously suggested, that T helper 2 (Th2) cells have a great impact on disease induction and perpetuation, aim of the present study was to assess whether Th2-related autoantibody subtypes, i.e. dsg3-reactive IgE, in addition to IgG4, are associated with clinical disease activity in PV. IgE autoantibodies had been previously identified by our group in a subset of PV patients and may be associated with eosinophilic spongiosis which is a common feature of early skin lesions in PV.

Levels of dsg3-specific IgE were quantified in the sera of 93 patients with defined clinical stages of PV, i.e. acute onset ($n = 37$), chronic active ($n = 42$) and remittent ($n = 14$) PV. Patients with acute onset disease showed highest titers of serum IgE autoantibodies, which were significantly lower in PV patients in remission. Furthermore, Th2-linked, dsg3-reactive IgE and IgG4 levels were strongly related to each other in patients with acute onset PV, but not in patients with chronic active or remittent PV. In contrast, Th1-regulated IgG1 autoantibodies were not significantly associated with IgE in any of the disease stages. To confirm the ELISA results, serum and tissue-bound IgE autoantibodies were also studied by immunofluorescence microscopy. Direct immunofluorescent staining of perilesional skin of a newly diagnosed, untreated PV patient revealed binding of IgE autoantibodies on the surface of epidermal keratinocytes. This finding was associated with eosinophilic spongiosis of the epidermis which is presumably regulated by Th2-driven cytokines. Moreover, IgE serum autoantibodies were detected by indirect immunofluorescence on monkey oesophagus as a substrate. Upon immunosuppressive treatment and clinical improvement of the PV patient, serum IgE autoantibodies were no longer detected by indirect immunofluorescencemicroscopy.

Thus, dsg3-specific IgE autoantibodies, in addition to dsg3-reactive IgG4, are related to acute onset PV supporting the concept that Th2-driven autoimmunity plays a fundamental role in the pathogenesis of PV.

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Relative IL-22 deficiency causes impairment of innate immunity in acne in versa

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Acne inversa is a chronic skin disease with unknown pathogenesis. In contrast to some other cutaneous inflammatory disorders such as psoriasis, AI patients suffer from bacterial infections of the skin lesions. We demonstrate here that the expression of antimicrobial peptides (AMPs), a major first line defense against bacterial skin invasion, was significantly diminished in AI lesions compared to psoriatic lesions. Further more, the AI lesions contained significantly lower expression levels of IL-22and IL-20, whereas the expression of IL-17A, IFN-gamma, and IL-1beta did not differ between both diseases. In reconstituted human epidermis IL-22 and IL-20upregulated the expression of diverse AMPs such as beta-defensin 2 and 3, and theS100 proteins S100A7, A8, and A9, and reduced the bacterial survival. The relativeIL-22 deficiency in AI was not linked to low cutaneous T-cell numbers or ROR gamma-t expression but might be caused by local presence of IL-10. In fact, cutaneous IL-10 expression was higher in AI and correlated negatively with IL-22expression, and moreover IL-10 inhibited IL-22 but not IL-17 production *in vitro*. The dearth of IL-22 in AI, in turn, may be the factor determining relative IL-20 deficiency since IL-22 upregulated IL-20 production of reconstituted human epidermis. We hypothesize that deficiency of IL-22 and its downstream mediator IL-20 contributes to the persistence of this chronic disorder.

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Correlation of disease activity in individual pemphigus patients with serum levels of autoantibodies to desmoglein 1 and 3 by the use of novel ELISA systems

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Pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are intraepidermal blistering skin diseases. PV is characterized by autoantibodies directed against desmoglein(Dsg) 3 and in patients with the mucocutaneous variant of PV also against Dsg 1. In contrast, in PF, only Dsg 1 is targeted. Here, ectodomains of Dsg 3 and Dsg 1 were recombinantly expressed in a human cell line (HEK293) and applied as authentic solid phases in ELISA test systems. Autoantibodies against Dsg 3 and/or Dsg 1could be detected in 100% of 71 PV sera and against Dsg 1 in 48 (96%) of 50 PF sera. Control sera from 401 healthy blood donors showed reactivity with Dsg 3 and Dsg 1 in 0.2% and 0.7%, respectively, and sera from 48 randomly selected patients with bullous pemphigoid, bound to these 2 targets in 2.1% of cases. No reactivity with Dsg 1 and 3 was detected in 21 patients with linear IgA disease. For both pemphigus variants, a statistically significant correlation between clinical severity and autoantibody levels was observed as demonstrated for 8 PV and 4 PF patients. In conclusion, the use of the ectodomains of Dsg 3 and 1 as target antigens expressed in a human cell line resulted in sensitive and specific ELISA systems for both diagnosis and monitoring of PV and PF.

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Pimecrolimus cream treatment repairs skin barrier structure more effectively than triamcinolone acetonide (TCA) cream treatment in atopic dermatitis

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In atopic dermatitis (AD) a defective skin barrier leads to the penetration of allergens into the skin, resulting in immunological reactions. In a prospective, randomized, double-blinded, right-left comparison study of 15 patients with mild to moderate AD, we investigated whether treatment with pimecrolimus 1% (PIM) or triamcinoloneacetonide 0.1% (TCA) cream twice daily for 3 weeks improves the skin barrier .TCA improved the clinical score, transepidermal water loss (TEWL) and epidermal proliferation more effectively, whereas improvement of stratum corneum hydration was slightly better after treatment with PIM. Transmission electron microscopy (TEM)studies of the barrier structure at the stratum granulosum / stratum corneum (SG /SC) interface showed much more regular lamellar body (LB) extrusion and regular SC lipid bilayers after treatment with PIM compared with TCA. Quantification of the TEM analysis revealed a lack of physiological lamellar bodies in AD lesional skin(32%; compared to healthy control). A significantly higher number of physiological lamellar bodies was found after 3 weeks of treatment with PIM (58%; $P < 0.005$) and physiological lamellar body extrusion and skin barrier structure formation occurred. A moderate increase in physiological lamellar bodies also occurred with TCA treatment (46%; $P < 0.05$), however, significantly less compared to PIM ($P < 0.05$).TCA improved clinical scores, TEWL and epidermal proliferation more effectively, whereas improvement of hydration was slightly better after treatment with PIM. The reduced TEWL in AD after treatment with TCA may be attributable to vasoconstrictive effects which reduce the influx of water into the skin. This indicates that TCA mimicsan improvement of the skin barrier in AD when only biophysical measurements are performed. In contrast, the TEM studies revealed that normalization of SC barrier structure occurred only after PIM treatment. This repair of the skin barrier hinders the entry of irritants and allergens into the skin and may prevent relapse of AD.

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An oil-in-water emulsion improves skin condition after irradiation for breast cancer

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The standard therapy for breast cancer is surgery followed by irradiation treatment, where radiodermatitis is a common side effect. There is no standard treatment for acute radiodermatitis. A widespread, yet unproven, theory proposes that skin cells better survive ionising radiation when the skin is dry and the cytosol contains less water. Severe dryness of the skin leads to inflammation, causing redness, pruritus and scaling, which are key symptoms of epidermal skin barrier dysfunction and stratum corneum dyshydration. In a prospective, controlled, randomized open-label study with 64 patients with radiodermitis, 32 patients were treated with an oil-in-water emulsion (WO 1932) and compared to untreated controls. Clinical evaluation included ONS radiation skin reaction scoring and a pruritus diary. We biophysically investigated stratum corneum hydration capacity (SCHC) and transepidermal waterloss (TEWL). In addition, quality of life (using EORTC QLQ-C30/B23, Skindex 29) and adverse events were monitored at day one (directly after termination of the irradiation therapy), at day eight (visit two) and between days 43 and 56 (visit three).Clinically, ONS scoring at visit three suggests that radiodermatitis was prevented in the cream-treated group (20% vs 41% in the untreated group, $P = 0.059$). The Skindex 29 did not show significant differences. Pruritus was significantly reduced after treatment with WO1932 compared to untreated patients. Reduced SCHC values in the irradiated field improved significantly faster in the cream treated area; at the third visit, SCHC had almost reached the values seen in the healthy skin of the contralateral breast (serving as control). TEWL was reduced in irradiated skin compared to normal skin. Application of WO1932 showed a tendency (not significant) toward normalization of TEWL. Quality of life assessment (EORTC QLQ-C30) revealed an advantage for the cream treated group in the evaluation of dyspnoea and role functioning. No adverse events were caused by the treatment regimens. In conclusion, treatment of radiodermatitis with an oil-in-water emulsion improves SCHC without harming the irradiated skin. Hydration of the epidermis prevents dry skin and itching, which improves patients' quality of life during irradiation treatment.

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Clinical effects of calcitonin infusions in systemic sclerosis -a retrospective study

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Apart from fibrosis and immunological features systemic sclerosis (SSc) ischaracterized by a vasculopathy which results among others in the characteristicRaynaud phenomenon. Calcitonin gene related peptide was shown to play an important role as mediator in sensory nerve in nervation as well as in circulation. As deficiency of this neuropeptide has been demonstrated in SSc, its therapeutic application has been advocated, though only rarely executed to a major extent. This treatment has been applied in our hospital for a decade and the results were evaluated in this retrospective study by monitoring the disease activity and organ involvement as well as serological parameters. 49 patients with diffuse ($n = 10$, 20%), limited ($n = 32$, 65%) or undifferentiated type of SSc ($n = 7$, 14%) have been followed-up for a median of 12.2 ± 10.3 cycles of intravenous calcitonin (100U per day for 10 days every cycle), each one with an interval of 3–6 months. Lung function (total lung capacity, CO-diffusion) improved in 14% of patients, remained stable in 46% of patients and deteriorated in 37%. Whereas creatinine clearance improved slightly during continuing treatment, it deteriorated again after stop of treatment. Cardiologic function (ECG, echogram) remained stable in 65% of patients. Serological parameters (CRP, BSR, ANA) changed without statistical significance or were unchanged. Furthermore patients were asked by a written questionnaire about an evaluation of their clinical response. 58% gave the overall judgement of 'good', 17% modest, 29% neutral, 4% 'bad'. The retrospective compilation of data is, however, a major constraint and limits further substantial conclusions. The indicated positive clinical effects of a continuous calcitonin treatment should be further examined in a prospective study.

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Multidimensional database for pruritus patients – statistical evaluation of clinical characteristics

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A descriptive, multidimensional database was programmed to survey the demographics, diseases and clinical characteristics of chronic pruritus patients. The database contains besides demographic data a large set of pruritus characteristics such as skin-condition, localisation, course and quality of pruritus. To date, the new installed database comprises 501 patients with chronic pruritus (240 m, 261 f, mean age 60.5 years). Next to itch, half of the patients describe the quality of the symptom to be mixed with burning (51.3%) or stinging (42.3%). 50.3% of the patients feel alleviation of itch by scratching, whereas itch increases through scratching in 36.3%,reflecting alloknesis, or turns into burning (22%). Nearly half the patients presented without scratch lesions (45.6%), about 19.3% showed single scratch lesions and34.6% had multiple scratch lesions including prurigo nodularis. The itch occurs mostly at daytime (48.5%) or in the evening (44.9%), respectively nights (42.9%).Pruritus trigger factors are especially pressure (39.1%) and touch (34.9%), followed by sweating (34.1%) and emotional stimuli (32.5%). Initially the pruritus was restricted in most patients to single areas (localized pruritus; 67.7%), which switched in the course of the disease into generalized pruritus (77.2%). Pruritus occurred mainly on the trunk (64.1%), the arms (71.5%) and the legs (71.9%). The itch intensity was measured by the visual analog scale from 0 to 10. The intensity was rated rather high by the patients since the mean value was 7.1 and the worst itch intensity in average 8.8. The data evaluation allows a deeper understanding of the course and characteristics of chronic pruritus and the patient perception of the symptom. Most interestingly are the different pruritus quality perceptions which may point to differentiated modulation of itch sensation. Recently, mechano-sensitivenociceptors (CMH) were demonstrated to represent another pathway for the itch sensation. Cowhage activates CMH and induces itch along with a stinging quality. It may therefore be speculated that the patients of our collective reporting on stinging quality (42.3%) have an involvement of the CMH cutaneous nerve fibers in itch induction. The impairment of the symptom by mechanical stimuli such as pressure and tough as observed in up to 40% of our patients further supports this hypothesis. An in-deep analysis of pruritus causes and therapeutic responses in these patients may lead to identification and characterization of new subgroups and pruritus pathways.

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Validation of dermaphot for the assessment of steroid induced skin atrophy (Validel)

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Steroid induced skin damage limits the long term usage of topical steroids in the treatment of atopic eczema. The typical sign of such skin damage is skin atrophy reflected in an increased transparency and telangiectasia. In atopic dermatitis the skin areas affected are often very thin (e.g. face, folds) and have an increased risk to develop earlier skin atrophy compared to other skin areas. Currently, there are no accurate and simple methods to measure the atrophogenic potential of different steroids.

The aim of the current study was to demonstrate that the Dermaphot® score is ableto differentiate the extent of skin atrophy (damage) after 3 weeks of topical steroid application with different skin atrophy and level of telangiectasia. Therefore, 36 adult healthy volunteers (18–60 years; male/female) were included in an investigator-blinded, randomized, intra-individual vehicle controlled multi-centre study. Skin parameters were assessed at baseline and after week 1, 2 and 3.Subjects were treated in a randomised manner on predefined areas on the lower arms with Pimecrolimus cream 1%, Mometasone furoate, Clobetasol propionate0.05% and Vehicle. The Dermaphot® score assesses the skin thickness (atrophy) and the telangiectasia in a score from 0–4. In addition ultrasound examination for skin thickness, optical coherence tomography and atomic force microscopy were performed at distinct study sites.

Data showed a direct correlation of the assessed Dermaphot® score and the ultrasound thickness measurements. At baseline all areas had a mean Dermaphot®score of 0.32 (= 100%). Areas treated with steroids showed an increase in Dermaphot® scores of 42.2% at week 1 and of 296.9% at week 3 compared to non-steroid treatment which exhibited a slight decrease in the scores with –3.7% at week 1 and –16.4% at week 3. Measured values were significant on a $P < 0.01$ level for the steroid versus non-steroid treatments. A strong decrease of skin thickness in the ultrasound measurements (20 MHz) corroborated the Dermaphot® results. Baseline values were 1014.5 μm (= 100%), while after 1 week areas treated with steroids showed a decrease in skin thickness of –10.7% those treated with non-steroids a decrease of –2.8%. At week 3 the decrease of skin thickness was enhanced to–18.5% in Clobetasol propionate and –13.8% in Mometasone furoate treated areas while the non-steroid treated areas showed a stable measurement of -2.1% in skin thickness. In conclusion our study shows for the first time that the Dermaphot® score can be used as a simple method to assess the athrophogenic potential of steroids.

Herewith, we showed that the Dermaphot® score is an easy, valid and sensitive new tool for early detecting and quantifying even subclinical steroid induced skin damage.

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Assessment of psoriasis burden with PRISM

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Psoriasis is a chronic frequent dermatologic disease having a big impact on patient's quality of life. It is supposed that the burden of suffering is an additional strong as pectin psoriasis disease. Pictorial Representation of Illness and Self Measure (PRISM) was recently developed to assess the burden of psoriasis.

The aim of the present study was to investigate, if there is a correlation between burden of disease and quality of life or if both reflect two different aspects in psoriasis. Secondary, correlation with disease severity parameters, e.g. Physicians Global Assessment (PGA), Psoriasis Area and Severity Index (PASI) and Body Surface Area (BSA) was also performed.

Therefore, PRISM was compared with Dermatology Life Quality Index (DLQI) for a total of 80 psoriasis patients recruited for this investigation. For assessment of PRISM, all patients received a white, A4 sized square plate and were asked to place a disk representing their psoriasis in relation to a disk representing their 'self' on it. The resulting distance between the two disks was recorded as a Self-Illness Separation (SIS) score. In order to validate retest-reliability for PRISM, the measurement was assessed a second time at the same day.

In addition demographic data and PGA, PASI, BSA scores were obtained at the same visit and also compared with PRISM.

The data shows a high correlation ($r = –0.923$) within repeated PRISM measurement. A middle to rather high correlation was also found for PRISM and DLQI ($r = –0.576$) and for PRISM and PGA. Whereas a middle to low correlation was found for PRISM and PASI ($r = –0.382$) and for PRISM and BSA ($r = –0.349$). No correlation was found for duration of psoriasis ($r = –0.147$)

We can conclude that for psoriasis, PRISM is a stable tool with a very high reliability. The moderate correlation between burden of suffering and quality of life demonstrates some common aspects of psoriasis. However, it seems that both elements reflect also different aspects of the disease and are not identical.

Compared to the severity of psoriasis, we can assume coherence to PRISM. Surprisingly, no relation between burden of disease and duration of psoriasis could be found.

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Patients' needs in pruritus treatment

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Aim: To determine treatment needs in pruritus from the patients' perspective.

Methods: Important treatment goals were determined by means of (1) an open questioning of $n = 50$ pruritus patients on their impairments and treatment goals and (2) subsequent expert discussions. The goals were implemented into the questionnaire 'Patient Benefit Index' assessing the importance the goals to the patients. N = 247 patients treated at the Department of Dermatology, Münster, or participating in clinical studies in Münster filled in the questionnaire. The percentage of patients to whom the goals were at least 'somewhat' important was calculated. Importance rates were compared between age groups (median split) and gender.

Results: 27 different treatment goals were included in the Patient benefit index.

53.8% of the patients filling in the questionnaire were female; the average age was 56.6 years.

The most frequent goals were to be free of itching (98.0%), to find a clear diagnosis and therapy (97.2%), to have confidence in the therapy (95.5%), to be less dependent on doctor and clinic visits (83.0%), to have no fear that the disease will become worse (81.0%), and to be able to sleep better (80.2%).

The goal of being able to wear all types of clothing was more frequent in women (59.2% vs. 45.0%, $P = 0.028$), whereas men more often aimed to be able to have more contact with other people (52.7% vs. 36.4%, $P = 0.011$).

Patients younger than 58 years more often wanted to be able to lead a normal working life (54.5% vs. 17.4%, $P < 0.001$) and a normal sex life (47.2% vs. 32.5%, $P = 0.020$). To patients aged 58 years or older, it was more important to be able to have more contact with other people (51.8% vs. 36.1%, $P = 0.015$).

Conclusion: There are various different treatment goals in patients with pruritus, some of which differ between older and younger patients and between women and men. These individual differences should be taken into account in treatment evaluation and in treatment choice in daily practice.

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Pseudomonas aeruginosa manipulates the cutaneous defense by repressinghBD-2 and psoriasin

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The contact between pathogenic microbes and skin represents the initial and essential step necessary for microbial colonization and invasion of the host. Albeit open for contamination, for the majority of microorganisms the human skin is unsuitable for a sustained microbial colonization. This resistance to colonization is mainly a function of the physical structure of the skin and its biochemical properties.

The biochemical defense barrier based mainly on the production of antimicrobial proteins (AMPs), small proteins of the innate immunity with antimicrobial activity towards various microbes. Activation of the innate immune response is achieved by the recognition of certain pathogen-associated molecular pattern (PAMP) resulting in an adequate defense-reaction of the host. Beyond the innate response triggered by PAMPs, pathogens have evolved strategies to subvert the immune response.

The ubiquitous bacterium *P. aeruginosa* occupies a diversity of ecological niches last but not least due to the versatile and non-stringent metabolic requirements. From this point of view it is not astonishing that *P. aeruginosa* considered to be a transient constituent of humans natural microflora and frequently found in human tissue infection. Adherence and colonization of surfaces by *P. aeruginosa* is accompanied with the formation of a self-produced extracellular matrix or biofilm, the opportunistic pathogen is embedded in. It is known that *P. aeruginosa* flagellin can induce theAMPs hBD-2 and psoriasin as well as proinflammatory cytokines as IL-8 inkeratinocytes. Thus we were curious to investigate, whether adherent growing *P.aeruginosa* secrete factors that repress AMPs and/or proinflammatory mediators to escape host recognition. For this *P. aeruginosa* were cultured under adherent conditions in minimal medium and bacterial supernatants were tested for their ability to repress hBD-2, psoriasin and IL-8-expression of keratinocytes stimulated with flagellin. As a result we found supernatants of P. aeruginosa able to repress hBD-2-and psoriasin-induction of flagellin- stimulated keratinocytes, while IL-8-induction was unaffected. Partial purification-experiments by treatment with methanol and chloroform as well as proteinase K-hydrolysis indicated that this factor is not a protein. Separation by size exclusion chromatography indicated a large molecular mass. Further investigations will identify this bacterial factor and enlighten the hBD2-and psoriasin-repressing mechanism in keratinocytes.

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Rapid detection of Burrelia burgdorferi DNA in superficial skin fluid fromerythema migrans

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Early diagnosis of erythema migrans (EM) can be uncertain. Moreover, antibody synthesis against *B. burgdorferi* may not be measurable before 3 weeks in about 25% of affected patients. A skin biopsy is not usually done. We describe the detection of B. burgdorferi DNA from a skin erosion of EM.

A 46-year old male patient presented with an erythema about 13 cm in diameter distal to his right patella. He also had a slightly tender lymph node in his right groin. The body temperature was normal. Laboratory examination of the blood including the blood count, C-reactive protein and anti-streptolysin titer were normal. IgM and IgG antibodies to *B. burgdorferi* were undetectable by ELISA. A central lesional skin area measuring about 1 cm was carefully scratched off with a rounded scalpel to obtain superficial tissue fluid which was then collected in a plastic mini tube. DNA was isolated and subjected to polymerase chain reaction (PCR). DNA specific for *B. burgdorferi* was clearly detectable as visualized on agarose gel. Antibiotic treatment with started with doxycycline treatment 200 mg daily per os for 20 days. The EM then completed healed. 3 weeks after the initial presentation, antibodies to *B. burgdorferi* could be detected as follows: IgG-ELISA 26 U/ml (positive) IgM-ELISA<20 U/ml (negative), IgG immunoblot 7 Index (positive), IgM immunoblot 8 Index (positive, VIsE, p41/I B. garinii, p41/I, B. afzelii bands). The treponema pallidumantibody test TPPA was negative.

Our findings show that the diagnosis of erythema migrans can rapidly be established in DNA specific for *B. burgdorferi* by PCR from superficial lesional tissue fluid obviating a skin biopsy.

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The increased expression of mouse β-defensin 3 in acute barrier disruption is mediated by TNF-α

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The physical permeability barrier and antimicrobial proteins of the innate immunesystem protect the skin against microbiological infection. We previously showed that expression of mouse β-defensin-3 (mBD-3) which is the ortholog of human β-defensin-2 (hBD-2), respectively, is stimulated by acute barrier disruption induced by tape-stripping or acetone treatment of mouse skin *in vivo*. Also, we found thatmBD-3 mRNA expression is induced in primary mouse keratinocytes by treatmentwith tumor necrosis factor-α (TNF-α) *in vitro*. We now asked whether the induction ofmBD-3 after skin barrier disruption depends on TNF-α signaling and whether TNF-α is the only cytokine involved in mediation of mBD-3 induction after skin barrier disruption. An anti-TNF-α antibody was injected in hairless mice (SKH-1) for blocking TNF-α activity 24 h before skin barrier disruption. 6 h after barrier disruption by acetone treatment, skin samples were taken for isolation of mRNA and for immunohistology. Realtime PCR analysis of samples with mBD-3 specific primers revealed a significant induction of mBD-3 gene expression by skin barrier disruption compared to untreated controls. Mice injected with an anti-TNF-α antibody before barrier disruption showed a significant decreased induction compared to control mice without anti-TNF-α antibody therapy. Immunohistology using a specific anti-mBD-3antibody confirmed these results on the protein level. In summary, we showed a crucial role of TNF-α in the induction of mBD-3 by skin barrier disruption. However, the anti-TNF-α pretreatment did not completely block the induction of mBD-3expression. This might be a hint for an involvement of additional cytokines in the gene induction of mBD-3 by skin barrier disruption.

P190

AP1 dependant repression of TGFα mediated MMP9 upregulation by PPARδ agonists in keratinocytes

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Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors, mainly implicated in the regulation of lipid and glucose homeostasis. In addition, PPAR agonists have been shown to control inflammatoryprocesses by inhibition of distinct proinflammatory genes. Several studies demonstrate that aberrant activation of the epidermal growth factor (EGF) and/or over expression of its ligand transforming growth factor (TGF)-α are key features of both neoplastic and inflammatory hyperproliferative epithelia. Matrix metalloproteinase 9 (MMP9) belongs to the set of genes that are effectively induced by TGFα in keratinocytes. Induced MMP 9 expression has been strongly linked to regenerative skin repair mechanisms and inflammatory skin diseases. We therefore explored whether the known anti-inflammatory effects of different PPARδ ligands are mediated in part through inhibition of TGFα-mediated MMP9upregulation. PPARδ agonists (e.g. L165, 041, GW501516) are found to potently inhibit TGFα-induced MMP9 expression by HaCaT keratinocytes, This inhibition is demonstrated both at the level of protein and mRNA MMP9 expression. Additional zymographic assays of culture supernatants show that PPARδ ligands significantly inhibit the catalytic activity of MMP9. As PPAR ligands do not interfere with expression and phosphorylation of the EGF receptor, we hypothesizedthat the inhibitory effects PPARδ agonists are mediated by suppressing the transcriptional activity of the MMP9 promoter. Transcriptional activation studies with deletional reporter gene constructs reveal that PPARδ agonists mediate their inhibitory effects via an AP1 binding site. EMSA analysis demonstrated TGFαinduced c-fos homodimer complex formation to this sequence is decreased by PPARδ agonist treatment, indicating that MMP9 gene expression is inhibited by repressing c-fos site-dependent DNA binding and transactivation. In conclusion, our data provide first evidence that TGFα-induced keratinocyte MMP9 expression is a valid target of PPARδ ligands, involving distinct mechanisms of AP1 dependant transcriptional repression.

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Role of neurotrophic factors & neuropeptides in healthy and atopic skin

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Atopic dermatitis (AD) is a highly pruritic, chronic inflammatory skin disorder characterized by eczematous lesions. The multifactorial disease is based on genetic predisposition. The observed changes of skin structure are exacerbated by endogenous and environmental stress factors, resulting in the disregulation of immunological reactions and promotion of inflammation. Increasing evidence suggests that neurotrophins secreted by skin cells are involved in the pathogenesis of AD, since AD patients show an increased density of nerve fibers in the epidermis. Using biochemical and molecular biology techniques we were able to measure neurotrophin secretion by non-neuronal skin cells. We hypothesize that higher nerve fiber density leads to an elevated level of the neuropeptide CGRP. Using Ca2+imaging and *in vitro* skin models we investigated the effect of CGRP on keratinocytebiology and on the structural development of the epidermis.

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In keratinocytes and melanocytes, but not in immune cells, IL-28 and IL-29 upregulate antiviral defense and proliferation regulating genes: potential role of a novel soluble IL-28 receptor

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Interleukin (IL)-28 alpha, IL-28 beta and IL-29 [also designated as type III IFNs] represent a new subfamily within the IL-10 - IFN cytokine family. They are closer to the IL-10-related cytokines in terms of gene structure, spatial protein structure and receptor usage, but display type I IFN-like antiviral and cytostatic activities forming the basis for e.g. IL-29 therapy currently under development for hepatitis C infection. However, many aspects of the IL-28/29 are still unknown. This study aimed to identify target cells and effects of these cytokines within two selected organs, the immune system and the skin. Among skin cell populations, keratinocytes and melanocytes, but not fibroblasts, endothelial cells, or subcutaneous adipocytes turned out to be targets. In keratinocytes IL-28 and IL-29 upregulated the expression of antiviral defense and proliferation regulating genes. However, blood immune cell populations did not clearly respond to even high concentrations of these cytokines despite clearIL-28/29 receptor (composed of IL-28R1/IL-10R2) mRNA and protein expression. Interestingly, immune cells expressed high levels of a short IL-28 receptor splice variant (sIL-28R1). Its characterization revealed a secreted, glycosylated protein that binds IL-29 with a moderate affinity (KD 73 nM) and was able to inhibit IL-29 effects. Our study suggests that IL-28/29 therapy in addition to patients with viral hepatitis should be suited for patients with verrucae, melanomas, and non-melanoma skin cancers, and would not be accompanied by immune-mediated complications like flu-like symptoms and induction/exacerbation of autoimmune disorders known from type I IFN application.

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Anti bacterial and antifungal effect of polyacrylate superabsorbers

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Introduction: Infection of the wound site can lead to the formation of a chronic wound. Pathogenecity and density of the colonizing microbes influence infection severity. However, when host defense mechanisms are impaired the risk of infection increases. Nosocomial infections have multiplied dramatically in the last years. Important pathogens of nosocomial infections are *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*. The spread of these pathogens can only be inhibited through consistent hygiene sanctions and preventive disinfectant actions. Polyacrylate-superabsorber containing wound dressings are able to take up large quantities of exudates while keeping the wound environment moist; an additional inhibition of bacterial and fungal growth would be a beneficial attribute. We have tested three different polyacrylate-superabsorber containing wound dressings according to the JIS L 1902for antibacterial and antifungal activity.

Material & Methods: *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* were chosen to monitor the antimicrobial effect. According to the JIS L 1902 norm samples of 400 mg of the polyacrylate-superabsorber containing wound dressings (Vliwasorb, Lohmann & Rauscher; Zetuvit plus, Hartmann; Sorbion sachet, Sorbion AG) were used for testing. The samples were incubated with the experimental pathogens(*Staphylococcus aureus*: 6,3×105cfu/mL, *Klebsiella pneumoniae*: 5,9×105cfu/mL,Pseudomonas aeruginosa: 9,1×105cfu/mL, Escherichia coli: 6,2×105cfu/mL und Candida albicans: 1,5×104cfu/mL) up to 24 h at 37°C under aerobic conditions.

Results: The polyacrylate-superabsorber containing wound dressings showed a strong inhibitory effect on *Klebsiella pneumoniae*, *Pseudomonas aeruginos* and *Escherichia coli*. They were also able to inhibit the growth of *Staphylococcus aureus* and *Candida albicans* significantly.

Conclusions: The polyacrylate-superabsorber containing wound dressing exhibit a distinct antibacterial and antifungal activity. Its use should help to treat wound infections by entrapment of the microorganisms in the forming gel on exudates' uptake and inhibition of their growth.

P194

Identification and assessment of diversity of clinical yeasts using MALDI-TOFMS

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Most pathogenic yeast species in dermatological clinical samples belong to the genera Candida, Pichia, Issatchenkia, Rhodotorula, and Trichosporon. However, the occurrence of other yeast species in dermatological samples has to be considered and rapid identification methods are required for reliable identification of these uncommon pathogens for adequate treatment.

We analysed yeast specimen from dermatological samples by MALDI-TOF MS, a technology for microbiological identification that is currently replacing classical methodologies in medical mycology. Yeast cells were prepared in for mass spectrometry directly from agar plates applying a standardized protocol and analysed automatically. Mass spectra of samples were matched against the database of SARAMIS (spectral archiving and microbial identification system) containing mass spectral data of a high number of reference strains. Among 104 blinded isolates, 98 could be identified as common yeast species, consistent with results from ID 32 Canalyses. For 80 these isolates, the mass spectral patterns showed similarities to those of frequently encountered Candida species, e.g. *C. albicans* and *C. glabrata*. Of the remaining isolates, several showed no similarity to Candida sp. in mass spectral patterns and could subsequently be identified unambiguously as species of the genera Pichia, Issatchenkia, Rhodotorula, and Trichosporon. For the remaining six isolates, mass spectral data of corresponding reference strains have not been available at the time of the first analyses but were collected consecutively. MALDI-TOF MS/SARAMIS has been successfully applied to the rapid and reliable identification of the majority of yeast isolates. The system further proved to be highly adaptive for the recognition and identification of rare fungal pathogens through database extension.

P195

RNase 7 protects healthy skin from *Staphylococcus aureus* colonization

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Staphylococcus aureus (SA) is one of the most common pathogens related with human skin infections. Despite the high carrier rate in the normal population skin is usually not infected by SA which is explainable by the ability of healthy skin to keep the bacteria within a limited number.

As part of its 'chemical barrier' antimicrobial peptides (AMP) are small cationic peptides with the capacity to kill various bacteria. Important skin-derived AMP are RNase 7, the S100A7 protein psoriasin and the human beta-defensins hBD-2 andhBD-3. The expression of these AMP in keratinocytes can be induced by contact with bacteria.

The aim of our study was to investigate the physiological relevance of RNase 7 in cutaneous defense against SA. Therefore standardized areas of human skin explants derived from plastic surgery were exposed to different concentrations of SA. After incubation for 2 h SA-treated skin areas were rinsed with an aqueous buffer solution and concentrations of RNase 7 was determined by specific ELISA. In addition, quantitative analysis of the remaining SA was performed. As a result, incubation with different concentrations of SA revealed increased secretion of RNase7. Killing of SA could be detected for all concentrations tested with killing rates up to more than 50%.

In another approach standardized areas of skin explants were incubated with SA after pre-incubation with a specific RNase 7-neutralizing antibody or with an irrelevant antibody. Pre-incubation with the RNase 7- neutralizing antibody resulted in an outgrowth of SA whereas an irrelevant antibody had no influence on the killing activity of the skin explants.

These data demonstrate that skin infected with living SA responds with an increased secretion of RNase 7 which contributes to limit the growth of SA thus indicating the physiological relevance of RNase 7 to protect human skin from SA infection. Future studies have to evaluate whether skin infections caused by SA may be associated with an impaired expression or function of RNase 7.

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Impaired Th1 and Th17 priming in mast cell-deficient KitW-sh/KitW-shmice

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Infection with the parasite Leishmania major leads either to self-healing cutaneous or systemic disease. To date, no effective vaccine exists. Previously, we demonstrated that mast cells (MC) significantly contribute to the control of parasitic skin infections by L. major using mast cell-deficient KitW/KitW-v mice. The c-kit mutations in KitW/KitW-v mice result in anemia and sterility. In contrast, mast cell-deficient KitW-sh/KitW-sh mice, bearing the W-sash (Wsh) inversion mutation, lack anemia and sterility. Adult KitW-sh/KitW-sh mice have been shown to exhibit a profound deficiency in MC in all tissues including skin, but normal levels of major classes of other differentiated hematopoietic and lymphoid cells. Thus, the latter mice may represent an additional, more relevant model for studying MC biology. KitW-sh/KitW-sh mice were infected with standard high dose (2×10E5) or physiological low dose inocula (1,000) of infectious stage metacyclic promastigotes of L. major mimicking natural transmission by the bite of a sandy fly. Similar to KitW/KitW-v mice, KitW-sh/KitW-sh mice showed significantly enhanced lesion development at several time points as compared to MC-competent mice (>2-folddifference), which was associated with increased parasite burdens and faster/higher parasite viszeralization. Interestingly, in the absence of MC, antigen-specific IFNγ release (critical for macrophage activation and parasite elimination) was significantly lower and Th2-associated IL-4 and Th2/Treg-derived IL-10 levels higher as compared to high dose-infected controls. Finally, the release of soluble Leishmania antigen-triggered IL-17A from CD4+ T cells was significantly lower in L.major-infected KitW-sh/KitW-sh mice. Thus, our data confirm prior *in vitro* data that indicated that MC-mediated maturation of dendritic cells (DC) leads to increased priming of naïve CD4+ T cells and Th education towards Th1/Th17. Thus, modulation of MC function *in vivo* may facilitate the induction of protective immunity against this important human pathogen.

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IL-23 is not required for Th17 induction in murine cutaneous leishmaniasis

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Previously, we have shown that IL-17A significantly contributes to disease outcome in murine cutaneous leishmaniasis. Protective immunity against L. major is critically dependent on the efficient development of IFNγ-producing Th1/Tc1 cells, as observed in genetically resistant C57BL/6 mice. BALB/c mice, in contrast, succumbto infection due to uncontrolled Th2/Th17 development. In addition to CD4+ T cells, IL-17 is also released by local neutrophils in leishmaniasis and persisting infiltration with neutrophils is associated with susceptibility. We now sought to determine the role of IL-23 in the pathogenesis of leishmaniasis. Increased amounts of IL-23p19were released upon infection of dendritic cells (DC) *in vitro*. We observed higher levels of IL-23 from L. major-infected BALB/c DC as compared to C57BL/6 DC both *in vitro* and *in vivo* which correlated with our previous observation of a higher frequency of Th17 cells in BALB/c mice. IL-23-deficient mice on a mixed C57BL/6 xxy background were infected with standard high dose (2×10E5) or physiological low dose inocula (1,000) of infectious stage metacyclic promastigotes of L. major mimicking natural transmission by the bite of a sandy fly. Lesion sizes inIL-23-deficient mice were significantly smaller from week 3 on post-infection as compared to those in control mice (e.g. 8.2 ± 0.5 vs 13.1 ± 1.1 mmE3 in week 3, $n = 5$ independent experiments, $P < 0.002$). Lesional parasite loads correlated with smaller lesion sizes ($P = 0.0025$). Interestingly, antigen-specific restimulation of lymph node cells revealed increased levels of Th1-associated IFNγ, but also significantly higher levels of Th2/regulatory T cell-derived IL-10 at several time points post infection.Th2-associated IL-4 production was unaltered. Finally, in the absence of IL-23, higher levels of IL-17A were produced. In conclusion, in contrast to our expectation, improvement of disease outcome in IL-23–/– mice was not correlated with decreased levels of IL-17. In addition, elevated levels of IL-10 in IL-23–/– mice did not lead to parasite persistence. Thus, in the model of cutaneous leishmaniasis, IL-23 may not represent the critical cytokine for the induction of Th17 priming, but may facilitate proper education of naive Th cells towards Th1.

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Staphylococcus aureus subverts cutaneous defense by d-alanylation of teichoic acids

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Staphylococcus aureus is a frequent skin pathogen associated with various skin diseases like impetigo, folliculitis, furunculosis and wound infections sometimes leading to systemic infections e.g. sepsis. Its pathogenicity suggests that it harbors special mechanisms to subvert the cutaneous defense system. Antimicrobial proteins are an important part of the cutaneous defense system because they are capable of killing a broad spectrum of microorganisms. Recently, it has been reported that a *S. aureus* mutant lacking D-alanine in the teichoic acids (dltA mutant) was highly sensitive to cationic antimicrobial proteins. Incorporation of D-alanine esters inteichoic acids reduces the negative surface charge of *S. aureus* leading to a reduced affinity to positively charged antimicrobial proteins.

Here we report that the *S. aureus* dltA mutant is also highly sensitive to cationic skin-derived antimicrobial proteins such as RNase 7 and human beta-defensin (hBD)-2. The functional relevance of these findings was shown by the higher sensitivity of the *S. aureus* dltA mutant towards stratum corneum extracts derived from heel callus. In addition, epidermal extracts derived from skin explants exhibited a higher killing activity against the *S. aureus* dltA mutant as compared to the *S. aureus* wild type.

Thus the incorporation of D-alanine esters in teichoic acids endows *S. aureus* with the capability to escape from host cutaneous defense through a diminished susceptibility towards skin-derived antimicrobial proteins.

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Immunogenicity of human papilloma virus (HPV) L2 antigen expression by virus-like particles (VLP)

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Human papilloma virus (HPV) vaccines are based on major capsid protein L1, self-assembled into virus-like particles (VLP). Immunization with L1-VLP induces high-titer and long-lasting neutralizing antibodies. The conferred protection against HPV infection and associated disease is predominately restricted to the vaccine HPV types (Cervarix, HPV 16 / 18; Gardasil, HPV 16 / 18 / 6 / 11). In contrast immunization with N-terminal peptides of minor capsid protein L2 can induce low-titer, cross-neutralizing, protective antibodies.

The immunogenicity of a cross-neutralization HPV16 L2 epitope was improved by surface-display on highly-ordered L1-VLP and further characterized in animal immunization studies.

The N-terminal HPV16 L2 peptide comprising amino acids 17–36 (RG-1) was genetically engineered into the DE-surface loop of bovine papilloma virus (BPV1) L1.The recombinant protein efficiently assembled into VLP. Immunization of NewZealand White (NZW) rabbits with native chimeric VLP induced higher L2-specificantibody titers in ELISA than vaccination with corresponding SDS-denatured proteins, using Freund's adjuvant. Chimeric VLP induced (cross)-neutralization to high-risk HPV 16/18/31/45/52/58, low-risk HPV11 and (skin cancer associated) beta-type HPV5 (titers of 50–10,000) in pseudovirion neutralization assays. Alum plus monophosphoryl Lipid A (MPL) adjuvanted VLP induced similar patterns of neutralization, in both NZW rabbits and Balb/c mice, albeit with 100-fold lower titers as compared to Freund's. Moreover, immunization with HPV16L1-L2 (17–36) chimeric VLP (plus alum-MPL) induced (cross)-neutralization of HPV16/18/31/45/52/58, HPV6/11, and HPV5 (titers of 50–100,000). Long-term evaluation of immunization with BPVL1-16L2 (17–36)/HPV16L1-L2 (17–36) chimeric VLP plus Alum-MPL in rabbits 6/9 months following the last injection showed about 10-fold decreased L2-specific neutralization titers, which were improved by additional vaccine boost.

Immunization with chimeric HPV16L1-L2 (17–36) VLP in adjuvant applicable for human use induces long lasting neutralizing antibody responses against mucosal high-risk, low-risk and beta (skin) HPV types.

P200

Peptides derived from the extracellular matrix exhibit antimicrobial activity against several microorganisms

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Laminins are a family of heterotrimeric extracellular matrix glycoproteins in the basement membrane of different tissues and are composed of alpha, beta, and gamma chains. In mammals, five different alpha chains, three beta chains, and three gamma chains have been identified, that assemble into 15 different laminins. Each alpha chain possesses a C-terminal globular domain, which can be subdivided in to the five domains LG1EUR"LG5. LG1-LG3 modules are connected to LG4-LG5 by a linker domain, which is known to be sensitive to proteolytic processing.

Here, we show that peptides derived from the human laminin alpha3, alpha4 and alpha5 chain of the LG4 module exhibit a dose-dependent antimicrobial activity against gram-positive and gram-negative bacteria. Furthermore, we show that these peptides can permeabilize the bacterial membrane and bind to bacterial DNA. Interestingly, the ability to kill the microorganisms correlated with their ability to bind to heparin. These data suggest that components of the extracellular matrix might playa role in the innate immune response of several epithelia by protecting the respective tissue from invading pathogens.

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The Dermcidin-derived antimicrobial peptide DCD-1L forms oligomeric structures and kills bacteria by interaction with the bacterial membrane

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Dermcidin (DCD) is an antimicrobial peptide, which is constitutively expressed in eccrine sweat glands. By post-secretory proteolytic processing in sweat the dermcidin protein gives rise to anionic and cationic DCD-peptides with a broad spectrum of antimicrobial activity. We could show that dermcidin-derived peptides inhibit significantly bacterial macromolecular synthesis (RNA, DNA, protein) within the first minutes without binding to microbial DNA or RNA. Recent structural analysis indicated that the anionic 48mer peptide DCD-1L forms ion-dependent oligomeric structures which are able to interact with the bacterial cell envelope and perturb the bacterial membrane structure. Further investigations by CD-spectroscopy and conductance measurements with artificial phospholipid membranes suggest thatDCD-1L is able to form small pores in the bacterial membrane which leads to ion efflux and bacterial death. These data show for the first time how an antimicrobial peptide present in human eccrine sweat is able to kill efficiently several types of microorganisms.

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Apoptotic Leishmania major in the inoculum support disease-promoting inflammation in the skins of BALB/c mice

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Objectives: It has recently been reported that the virulent inoculum of Leishmania major (L.major) pro-mastigotes conventionally used for infection experiments contains about50% of apoptotic parasites. The depletion of apoptotic parasites lead to a reduced infectivity in susceptible BALB/c mice *in vivo*. Our study aims to analyze the detrimental effect of apoptotic leishmania on the immune response in the skin.

Methods: We separated viable and apoptotic L. major by magnetic cell separation and infected susceptible BALB/c mice subcutaneously in the hind foot pad with 1 million viable parasites. Cytokines, cell markers and FcγRs expression in the skin was analysed using real-time RT-PCR, leishmania-specific and total serum IgG subtypes were determined by ELISA. Statistical significance between groups was determined by the two-tailed nonparametric Mann-Whitney test for unpaired samples.

Results: We found that apoptotic parasites in the inoculum promoted disease progression and increased IL4 and IL10 mRNA levels 6 weeks after infection, while IFNγ levels remained unchanged. Furthermore, apoptotic leishmania enhanced serum IgG1 and the expression of the IgG1-binding Fcγ-receptor 3 in the skin. Disease, cytokine mRNA production and serum IgG1 after 6 weeks could be reduced by removing apoptotic leishmania from the inoculum.

Conclusion: Apoptotic L. major parasites in the inoculum have a strong influence of disease-promoting inflammation in the skin.

P203 (V30)

Delivery of flagellin by *Pseudomonas aeruginosa* rhamnolipids throughout the skin barrier induces an innate immune response

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Human skin represents the first barrier against invading microbes and foreign molecules. The innate immune function is due to the hierarchical structure of the skin. This physical barrier is complemented by a biochemical barrier of antimicrobial substances like antimicrobial peptides (AMP). Although not belonging to the typical resident skin micro-flora, *Pseudomonas aeruginosa* is commonly found in different locations of healthy individuals. This flagellated bacterium normally lives harmless on the surface of human skin, but can infect any tissue it comes in contact with. Survival of *P. aeruginosa* on surfaces is often connected with a switch from the motile planktonic way of life to a structured community of bacterial cells enclosed in a self-produced polymeric matrix - the bio-film. Rhamnolipids, bio-surfactants produced by *P. aeruginosa*, play an important role in the initial phase of this bio-film formation. Since the human skin is an efficient barrier against invading microorganism and possesses very low permeability to the movement of foreign molecules across it, we investigated a possible role of rhamnolipids to deliver the pathogen associated molecule flagellin through the skin barrier. Flagellin was isolated from *P. aeruginosa*. We could detect a flagellin-induced increase of interleukin-8 and psoriasin in cultured keratinocytes in a dose and time dependent manner. Immunohistochemical analysis of the AMP psoriasin expression in full thickness skin biopsies revealed a strong increase of immunoreactivity of psoriasin in the upper epidermis using flagellin together with rhamnolipids, while rhamnolipids alone did not induce psoriasin expression. A flagellin deficient *P.aeruginosa* strain failed to induce psoriasin expression in the *ex vivo* model in comparison to its parental strain. Different clinical isolates from *P. aeruginosa* exhibited rhamnolipid expression. Since rhamnolipids are anionic amphiphilic molecules with detergent properties, we investigated in what extent chemical detergents can substitute the function of rhamnolipids in the flagellin delivery across the upper skin layers. Co-stimulation of flagellin together with the anionic sodium deoxycholate, the zwitter-ionic CHAPS and, the cationic hexadecyltrimethyl ammonium bromide revealed no visible psoriasin expression. Solely analyses of skin stimulated with TWEEN 20 along with flagellin resulted in a visible increase of psoriasin expression similar to that obtained for rhamnolipids and flagellin. Here we provide evidence that the opportunistic pathogen *P. aeruginosa* is recognized by human skin during its settlement by flagellin in combination with rhamnolipids, which leads to the induction of an antimicrobial response. This might be the reason that *P. aeruginosa* is only a transient constituent of humans natural micro-flora.

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Role of interleukin-22 (IL-22) in murine cutaneous leishmaniasis

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Healing of Leishmania major infections in resistant mice (e.g. C57BL/6) is based onTh1/Tc1 immunity, since IFNγ secretion of CD4+ Th1 and CD8+ Tc1 cells is critical for macrophage activation and parasite elimination. In contrast, Th2/Th17 induction leads to systemic disease and susceptibility as observed in BALB/c mice. In this model, IL-17A released from CD4+ T cells and neutrophils contributes to parasite persistence. The role of Th17-associated IL-22 in L. major infections is unclear. IL-22is presumed to play an important role in innate pathogen defense by producing antimicrobial peptides. Here, we analyzed the role of IL-22 in experimental cutaneous leishmaniasis. In draining lymph node (LN) cells of infected C57BL/6 and BALB/c mice, we detected elevated levels of IL-17A from BALB/c cells upon antigen-specific re-stimulation, whereas IL-22 production was significantly higher (~3-fold) in LN cells from C57BL/6 mice. C57BL/6 mice deficient for IL-22 exhibited significantly decreased lesion development after intra-dermal, low dose infections with L. major mimicking natural transmission of the parasite by the bite of a sand fly as compared to wild type C57BL/6 mice. In parallel, lesional parasite burdens at the side of infection were significantly smaller ($P = 0.004$, $n = 3$ independent experiments) in IL-22–/– mice 9 weeks post infection, whereas antigen-specific cytokine production (IFNγ, IL-4, IL-10 and IL-17A) of draining LN cells was unaltered. Finally, in Leishmania-resistantC57BL/6 mice, the majority of IL-22 appeared to be secreted by γ/δ T cells after re-stimulation with dendritic cells pulsed with soluble Leishmania antigen (SLA) or infected with L. major as compared to CD4+, CD8+ T cells or neutophils. On going studies will aim to further characterize the source of IL-22 and the mechanism of action by which IL-22 promotes disease development in C57BL/6 mice. A detailed understanding of the role of the various Th17-derived cytokines in cutaneous leishmaniasis will aid the development of vaccination strategies against this important human pathogen.

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FAN-associated migration of inflammatory cells to sites of skin infections contributes to protective immunity

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Prior work with TNF receptor (R) p55–/–, or p55–/–/p75–/– double-deficient mice identified TNF-α/TNF-R signaling as an important pathway for the induction of protection against murine cutaneous leishmaniasis. Here, parasite clearance was delayed and non-resolving lesions in p55–/– mice were correlated to impaired apoptosis of inflammatory lesional cells. We now studied the role of FAN (factor associated with neutral sphingomyelinase activation) in L. major infections, a molecule mediating TNF-induced cdc42 activation and actin re-organization. FAN–/–mice showed defects in their cutaneous barrier repair, but so far no role for regulation of immune responses was identified. FAN–/– mice were infected with standard high dose (2x10E5) or physiological low dose inocula (1,000) of infectious stage metacyclic pro-mastigotes of L. major mimicking natural transmission by the bite of a sandy fly. Interestingly, FAN-deficient mice (on a Leishmania-resistant C57BL/6background) showed significantly worsened disease outcome with more severe lesion development over the entire course of infection starting early in week 2–3 (e.g.3.8±1.0 vs. 20.6±3.9 mmE3 in week 9, $P = 0.0001$, low dose model). Full lesion resolution was not observed until wk18. Lesional parasite loads were high and unaffected by the absence of FAN at earlier time points of infection, whereas in week 9 post infection (low dose model), significantly higher parasite burdens were determined in FAN–/–mice. Interestingly, antigen-specific cytokine responses of lymph node (LN) cells were unaltered and showed a Th1-dominant profile. However, we detected an impaired proliferation of antigen-specific CD8+ T cells upon stimulation of LN cells (CFSE labeling), CD4 priming was unaltered. The draining LN contained a significantly higher percentage of CD4+ T cells. In skin, the inflammatory infiltrate was dramatically altered and fewer F4/80+ macrophages, CD11c+ DC, CD49b+ NK cells and CD3+ (CD4+ and CD8+) T cells immigrated into sites of infection. The number of inflammatory neutrophils was unchanged. In addition, release of neutrophil-recruiting IL-6 from draining LN cells was normal. In conclusion, our data suggest that proper recuitment of innate effector cells to the skin is dependent on FAN. Future experiments will elucidate the contribution of FAN to the migratory properties of these inflammatory cells in L. major infections in more detail.

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Control of anti-Leishmania immunity: Suppression by epidermal Langerhans cells versus healing promoted by CD11c+ dendritic cells

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As there is still no vaccine available against Leishmania infections and considering the critical roles of (skin) dendritic cells (DC) as regulators of the anti-Leishmania immune response, DC are attractive targets for immunotherapy. To this aim, it is important to understand the precise contribution of particular DC subsets in the course of leishmaniasis. Defense mechanisms against pathogens are exerted by different DC subsets, including dermal DC (d DC) and Langerhans cells (LC).Knock-in mice expressing a diphtheria toxin (DT) receptor (DTR) cDNA under control of the langerin promoter were generated to study the *in vivo* dynamics and functions of Langerin+ DC in general and LC in particular. In this study, we analyzed the role of LC and dermal DC in murine experimental leishmaniasis by their inducible ablation *in vivo*. Upon physiologically relevant low-dose infection with L. major (1,000 parasites), mice selectively depleted of LC developed significantly smaller ear lesions and decreased lesional parasite burdens, which correlated with reduced numbers of lesional CD4+/Foxp3+ regulatory T cells (Treg) as compared to control mice. This was accompanied by increased IFNγ/IL-4 and IFNγ/IL-10 ratios upon antigen-specific re-stimulation of lymph node cells prepared from LC-depleted mice. In an independent experiment, we utilized CD11c-DTR (donor) - C57BL/6 (recipient) bone marrow (BM) chimeras to deplete CD11c+ DC, but not the radio-resistant LC. During low dose infection with L. major, these BM chimeras developed significantly exacerbated disease with larger lesion volumes as compared to PBS-treated controls. In conclusion, our data reveal a suppressive role of LC in leishmaniasis by Treg-derivedIL-10 leading to attenuated Th1-responses. In contrast, CD11c+ d DC are required to promote the induction of efficient protective immunity. Thus, future immune intervention strategies will aim to circumvent LC and selectively target d DC for the development of vaccines against this important human pathogen.

P207

Study of the influence of polihexanide on *Staphylococcus aureus* by microplate-laser-nephelometry

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Introduction: Infection is the main cause of delayed healing in surgical, traumatic and burn wounds, and may lead to the formation of a chronic wound. The severity of infection depends on the pathogenecity and density of the colonizing microbes. However, the risk of infection increases when host defense mechanisms are impaired. In the last years, nosocomial infections have been found to multiply dramatically. *Staphylococcus aureus* is one of the most important pathogen of nosocomial infections and is common complication during the treatment of chronic wounds. Rather than relying on just debriding and cleansing the wound, additional therapeutic strategies are commonly applied in an attempt to prevent infection. Therefore, wound dressings combined with antimicrobial agents are increasingly utilized in the treatment of critical colonized or infected chronic wounds. Polihexanide is regarded first choice for the treatment of chronic wounds because of its good skin tolerance beside its antimicrobial effects. Furthermore, a positive influence of polihexanide on wound closure was observed in individual clinical cases. Although, antiseptics have a lower potency to induce bacterial resistance than antibiotics, concerns have been expressed regarding the overuse of antiseptics and the emergence of bacterial adaptation, particularly in the clinical environment. Clinical evidence for silver-resistant bacteria has been observed in hospitals where silver salts are used for disinfection. Hence, we have used an experimental system using microplate-laser-nephelometry to test the adaptation capacity of *Staphylococcus aureus* to continued treatment with polihexanide and silver nitrate.

Material & Methods: *Staphylococcus aureus* was incubated with polihexanide (0.1–0.6μg/mL) and silver nitrate (1-40μg/mL). Bacterial growth was investigated by laser nephelometry (NephelоSTAR, BMG Labtech, and Germany). IC50 concentrations of the antiseptics were determined. Subsequently, the microorganisms were repeatedly incubated with the respective IC50 concentration (polihexanide: 0.2μg/mL, silver nitrate: 5μg/mL) for 100 days. Influence of the continued treatment was determined by calculation of the IC50. Additionally, a polihexanide and a silver containing wound dressing (Suprasorb X + PHMB, Suprasorb A+Ag) have been tested according to the JIS L 1902 for antibacterial and antifungal activity using untreated and treated *S.aureus*.

Results: IC50 concentrations of polihexanide increased only slightly over time ($m = 0.002$). In contrast, a dramatic increase of the IC 50 concentration was observed for silver nitrate ($m = 0.087$). Furthermore, the tests of antimicrobial activity against *Staphylococcus aureus* according to the JIS L 1902 revealed a decrease in the affectivity of the silver containing wound dressing. The polihexanide containing wound dressing showed the same microbial growth reduction in this test using treated and untreated *S. aureus*.

Conclusions: Increasing use of antiseptics may result in bacteria that are less susceptible. Silver dressings have been shown to be bactericidal and fungicidal; however, a possible development of resistance to silver has been suggested. In the present study the IC50 concentration for silver nitrate was found to increase with repeated treatment of *S. aureus*. On the other hand, polihexanide showed a much lower potency under these test conditions to induce adaptation in *S. aureus*. Therefore, it seems to be an appropriate antimicrobial substance in wound dressings for treating chronic wounds because of its low cytotoxicity, good skin tolerance and positive influence on proliferation.

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A new pigmented human 3-dimensional epidermis model integrating primary melanocytes

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For cosmetic and chemical industry the development of novel cosmetics for skin tanning or bleaching are important issues. The active ingredients used in these products have to be tested for efficacy and product safety. The aim of this study was to create a 3-dimensional epidermis model harboring melanocytes at the basal cell layer, their physiological localization. Based on the technology of the epidermal skin test (EST1000) we tested different ratios of melanocytes and keratinocytes for the production of the skin models. For testing effects on skin pigmentation an important feature of that epidermis models is the feasibility of cultivating the models for at least ten days after application of test substances without loss of viability. As endpoint analyses we determined the physiological behavior of the newly developed epidermis model containing melanocytes (MST). We confirmed the pigment synthesizing activity by analyzing the melanin content. The viability was assessed by standard MTT-test. We addressed the localization of the melanocytes by immunohistochemistry using the HMB45 antibody. We could demonstrate a constant high viability for about 23 days of culture. High melanin content is displayed at an optimum ratio of melanocytes and keratinocytes. The immunohistochemical analyses showed the localization of melanocytes in the basal cell layer.

P209

Dimethylfumarate contributes to overcome tumor-induced tolerance of melanoma cells by inhibiting indoleamine2, 3-dioxygenase

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The enzyme indolamine2, 3-dioxygenase (IDO) is part of a molecular mechanism which contributes to tumor-induced tolerance. IDO catalyses the rate-limiting step of tryptophan degradation along the kynurenine-pathway. Both, the reduction of local tryptophan concentrations and the production of L-kynurenine contribute to the immuno-suppressive effects of IDO. Studies of serum concentration of tryptophan and L-kynurenine indicate that IDO is chronically activated in a number of patients with cancer and that IDO activation correlates with more extensive disease. In primary skin melanomas it was shown, that expression of IDO increased with melanoma progression. Dimethylfumarate (DMF) is the essential ingredient of a registered, well established drug product used for the systemic treatment of psoriasis. Recently, it was shown in a phase-II clinical trial that DMF as mono-therapy is beneficial in the treatment of relapsing-remitting multiple sclerosis. Interestingly, orally administered DMF reduced melanoma growth and metastasis in severe combined immuno-deficient mouse models. Furthermore, preliminary data showed that DMF potentiate the therapeutic effect of dacarbazin treatment of malignant melanoma in an animal model. These effects could partially be explained by DMF as an inhibitor of cell proliferation and inducer of apoptosis. The effects of DMF on IDO-activity in melanoma cells have so far not been investigated. Therefore, melanoma cells were treated with different concentrations of DMF for 24h and stimulated with 100 ng/ml interferon γ. The IDO-activity was determined by measuring the concentration of tryptophan and L-kynurenine in the culture medium employing a HPLC-technique. Since it is assumed that DMF could exert its effects by modulating the intracellular GSH-content of cells, melanoma cells were harvested after 1h and 24h incubation and levels of GSH were measured via an enzymatic method. The results of the study show that the treatment of melanoma cells with DMF results in a slightly lower IDO-activity. Intracellular GSH levels of melanoma cells were significantly decreased after 1h incubation with DMF. The extent of GSH degradation was linked to the concentration of DMF in a linear fashion. After 24h GSH levels were increased to the original level. The results of this study imply that DMF could affect the IDO-activity in melanoma cells by depleting the intracellular GSH-content. By affecting the IDO-pathway DMF could serve to overcome tumor-induced tolerance of the immune system resulting in a more effective melanoma-therapy and at least in part explain the adjuvant effect observed in animal models.

P210

Everolimus, a rapamycin derivative, suppresses collagen synthesis *in vitro* and *in vivo* - New perspectives for the treatment of fibrotic skin diseases

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Treatment of fibrotic diseases including those of the skin remains a therapeutic challenge. Here, we investigated the effect of the mTOR inhibitor everolimus on collagen synthesis of human dermal fibroblasts as well on skin and lung fibrosis in the bleomycin mouse model. At present, everolimus is approved for the clincial use as a preventive treatment of allograft rejection in transplant patients. Everolimus dose dependently suppressed bFGF induced metabolic activity and proliferation of human dermal fibroblasts *in vitro*. The drug also significantly attenuated TGF-beta1-and bleomycin-induced collagen synthesis *in vitro*. These effects were closely related to inhibition of mTOR activity of human dermal fibroblasts as measured by phosphorylation of S6RP. However, everolimus did not induce the autophagy markers Beclin-1 and LC3B-II. Importantly, everolimus administered orally twice daily at doses used for the prevention of allograft rejection potently suppressed skin and lung fibrosis in adult mice exposed to bleomycin. In summary, these data highlight a novel treatment avenue for the treatment of fibrotic skin (and lung) diseases with them TOR inhibitor everolimus.

P211

Laser-stripping of the stratum corneum to enhance topical 5-aminolaevulinicacid delivery for photodynamic therapy: continuous vs. fractional ablation

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Limited uptake or penetration of topically applied 5-aminolaevulinicacid (ALA) may account for highly varying response rates in PDT. We investigated the skin penetration after application of either a 20% ALA cream or a 20% aminolaevulinicacid solution (Levulan® Kerastick TM) on laser-stripped stratum corneum (SC) using an *ex vivo* porcine skin model by means of fluorescence detection of protoporphyri-IX (PpIX). Two different Erbium:YAG laser systems (2,940 nm) were used to partially ablate the SC: continuous ablation (fluence rates ranging from 0.5–1.5 J/cm^2) and fractional ablation (fluence rates ranging from 4–24 J/cm^2). The latter is a relatively new technique, also termed 'fractional ablative photo thermolysis.' Further objectives were the assessment of the efficacy and safety of Er:YAG laser systems, in particular, the optimal laser exposure parameters to maximize percutaneous permeability and minimize epidermal alterations. Fluorescence microscopy was used for detecting PpIX induced fluorescence as a marker of ALA penetration and distribution. An optimal parameter for continuous ablation without damage of the underlying dermis was 1.0 J/cm^2. After continuous ablation with 1.0 J/cm^2, followed by 4h incubation with lipophilic ALA cream or the aminolaevulinic acid solution, mean fluorescence intensity (MFI) of PpIX were enhanced 13.8-fold and 1.5-fold, respectively. An optimal parameter for fractional ablation without damage of the underlying tissue was 4 J/cm^2. MFI of PpIX was enhanced 2.5-fold or 7.3-fold after fractional ablation with 4 J/cm^2 and incubation with Levulan® KerastickTM or lipophilic ALA cream for 4h, respectively. In laser-stripped skin, PpIX fluorescence as a parameter for ALA penetration was detected earlier and reached deeper epidermal layers than in untreated skin. Continuous laser-stripping seems to induce higher PpIX fluorescence levels than fractional ablation. This method offers a promising new tool for enhancing ALA penetration in PDT without damaging the underlying epidermal tissue necessary for PpIX induction. A further advantage would be shorter incubation times when treating superficial lesions, such as actinic keratoses, superficial basal cell carcinomas, and Bowen's disease. Fractional ablative photo-thermolysis in combination with PDT may be a new tool for treating photo-damaged skin.

P212

Variation in the effectiveness of UV induced 25OHD synthesis depending on the anatomical location of the epidermis

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About 95% of the effective solar UV irradiance (mid latitudes) to convert pro-vitamin D to pre-vitamin D in human epidermis is concentrated in the wavelength range298...315 nm. Furthermore, in this range its spectral effectiveness is similar to the UV erythema action spectrum. Therefore, in our studies on UV-induced vitamin D synthesis the individual UV exposure of the included volunteers was applied with respect to their minimal erythema doses (MED). The increase of the 25OHD serum level was roughly independent e.g. from the spectrum of the UV source and from the UV skin type. Hence, the UV erythema sensitivity is an indicator of the efficiency to produce 25OHD after UV exposure of the skin in the investigated persons. In a current research project*) we investigate the UV exposure conditions needed to realize an optimal vitamin-D-status (25OHD3 ≥ 30 ng/ml) throughout the year. The aims are recommendations to the public on optimal daily solar exposure times and exposed skin areas. This background leads to the question: Is the effectiveness of UV-induced vitamin-D-production independent of the anatomic location of the skin? Former investigations found a distinct effect of anatomic location on UV erythema. We hypothesized that there is a dependence of the effectiveness of the UV-induced vitamin-D-production from the anatomic location of the skin. Currently, no variations are assumed in this effectiveness. To test our hypothesis we investigated the effectiveness of the UV-induced vitamin-D-production of dorsal hand/forearm, calf, thigh, face, abdomen, back (skin type-II n = 10; skin type-III n = 10).For one location after the other the 25OHD serum level before and after three serial UV exposures (70% of the individual MED of volunteers back) was determined. We found distinct differences in the effectiveness of the UV-induced vitamin-D-production, rising up to 400% between the investigated anatomical locations (e.g. between face and dorsal hands). The relationships of the effectiveness between the locations of the skin are comparable to the MED relationships between these locations. There were only marginal, with no significant differences between the skin types. In conclusion we can state: For the solar UV induced 25OHD3 increase the different vitamin D production effectiveness of the skin has to take into account and, moreover, the (seasonally changing) body distribution of the solar UV exposure affecting the vitamin-D-production in the skin areas of the different anatomical locations. *) supported by Federal Ministry for the Environmental, Nature Conservation and Nuclear Safety / Federal Office for Radiation Protection (Support-N°: StSch 4538)

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Polycyclic aromatic hydrocarbons in black tattoo inks, singlet oxygen generation and risk assessment

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In recent years, there has been an increase in the placement of body art involving puncturing of the skin such as tattoos, permanent make up and body piercing. For black tattoo inks, the chemical nature is based on carbon black (CB). CB is known to act as a significant strong sorptive phase for polycyclic aromatic hydrocarbons (PAHs). In addition, PAHs are suspected to generate reactive oxygen species (ROS) under UV irradiation, such as singlet oxygen that affects DNA integrity. To assess the health risk of tattooing and the light interaction with the respective black tattoo inks, it is important to determine the concentration of PAHs in tattoo inks. Thus, we developed an ultrasonic-assisted extraction method using a mixture of benzene/acetone, heat and centrifugation. We purchased 19 inks from different tattoo suppliers in Europe and US and the inks were delivered as 'ready to use'. To identify the PAHs using the Internal Standard method by LC-MS, we selected 20PAHs as reference, which are frequently found in environment, food or cigarette smoke. These PAHs are listed by the US EPA due to their toxicity and carcinogenicity or Scientific Committee on Food in Europe. The total amount of PAHs in the tattoo suspensions was up to 201 μg per mg dry tattoo ink. In addition to PAHs, we found a high amount of phenol with up to 385 μg per mg dry black tattoo ink. Phenol is toxic and can damage kidney and the central nervous system. Then, we determined the quantum yield of singlet oxygen generation for each PAH using luminescence detection. The quantum yield of the different PAHs ranged from 0.46 to 0.82, which is higher than for photo-sensitizers in photodynamic therapy. Human keratinocytes were incubated with PAHs that have been extracted form 1 mL black tattoo suspension. After irradiation with broadband UVA, the cell viability (MTT-assay) decreased significantly. Furthermore, DNA-PAH interactions after UVA irradiation, such as formation of 8 oxo dG, are under investigation. In conclusion, black tattoo inks contain high amounts of PAHs and phenol. The PAHs generate singlet oxygen, which in turn affects cell viability and may probably cause DNA damage.

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P214

Photodynamic fungicidal effect of a two-fold positive charged porpyhrine-derivative and visible light against Candida albicans

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In recent decades, the emergence of fungal infections has increased considerably due factors such as the use of invasive procedures, immunosuppressive medications, broad-spectrum antibiotics, neutropenia and HIV infections. Opportunistic pathogens from the genera Candida can cause mucosal and skin infections and evolve to invasive fungal infections, especially in immuno-compromised patients. In these cases, invasive fungal infections are associated with high morbidity and mortality. A new approach to treat microbial infections of the skin uses light in combination with a photo-sensitizer (PS) to induce a phototoxic reaction similar as in PDT for skin cancer. Recently we could show that a new two-fold positive-charged porphyrine-derivative (PS2+) yielded a 4-log10 decrease of viable cell number (≥99.99% killing efficacy) of different MRSA strains upon illumination. The goal of this study was to investigate the photo-toxicity of this new porphyrine-derivative PS2+ against the leading opportunistic pathogen *Candida albicans.* Cellular uptake of the PS2+ was detected by fluorescence spectroscopy.5x10^7 planktonic *Candida* cells were incubated with different concentrations (0–5μM) of the PS2+ for different incubation times (0–15 minutes) upon irradiation with 13.4 m W/cm^2. Killing of *Candida albicans* mediated by the PS2+ and blue light (λ em: 418 ± 20 nm) was concentration and light dose dependent, achieving upto ≥99.999% (≥ 5log10 reduction) efficacy of Candida cell killing. Fifteen minutes incubation of Candida with 1 μM of PS2+ resulted in a killing efficacy ≥99.9% upon irradiation. Light doesn't have alone any effect on the cell viability. No dark toxicity of PS2+ was observed. In clinical practice antimicrobial agents are less effective against bio-film growing pathogens as compared to planktonic growing cells. Therefore a *Candida* bio-film model was established additional to evaluate PS2+ toxicity in a more realistic *in vivo* situation. Significantly higher concentrations of PS2+ were necessary to kill ≥99.9% of bio-film growing Candida cells.

Overall this study provides useful information that combination of a two-fold positive charged porphyrine-derivative and blue light may be a potentially powerful tool for efficient killing of bio-film growing opportunistic fungal cells.

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In vitro study on light dosimetry variables of five different intense pulsed light systems for 5-aminolevulinic acid photodynamic therapy

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Incoherent light is frequently used for PDT of topical skin lesions, because of the broad emission spectrum of the lamps and the general maintenance of these lamps is easier and cheaper. Effectiveness of a given light source depends on several factors, such as radiation energy, wavelength range, tissue transmission and the absorption properties of the photo-sensitizer. Recently, intense pulsed light systems (IPL) were developed for different dermatological indications like rejuvenation, port-wine stains and hair removal. These devices contain flash lamps with short-pulse duration of up to 100 ms and different cut-off filters, which allow using the wavelength range optimal for light penetration and excitation. IPLs are widespread in dermatological practize having different emission spectra and pulse durations. In this study out of the various IPL-systems on the market we have chosen five widely used IPLs to evaluate their availability to be used in PDT. Emission spectra and optimal light parameters to kill 50% of keratinocytes upon incubation with 5-ALA*in vitro* were determined. The used IPLs contain different cut-off filters as follows: IPL-I-550 nm; IPL-II-500 nm and II-550 nm; IPL-III-400 nm and III-550 nm; IPL-IV-560 nm; IPL-V-515 nm and V-535 nm. A standard LED light source (peak 630 nm) was used as a control. The IPL-III-400 using light doses in the range of 8.4J/cm^2–67.2 J/cm^2 showed the most efficient killing activity up to 100%. The IPL light doses, which were needed in order to achieve the EC 50 value upon illumination ranged between 80.1 J/cm^2 (IPL-III-550) and 311 J/cm2 (IPL-I-550). An exception was the IPL-III-400, which already approached the EC50 value using a light dose of36.4 J/cm^2, which is in the same range as the LED light. All five IPL light sources are capable to excite protoporphyrin IX, the actual photo-sensitizer in 5-ALA PDT, but produce less PDT activity than the standard LED light source regarding the killing efficacy of keratinocytes (EC50 value). Overall the results showed that the measured emission spectra of the tested IPLs determine the efficacy of the photodynamic killing of keratinocytes *in vitro*. However, IPLs of different manufacturers which had similar cut-off filter specification did not show similar efficacy. In conclusion the tested IPLs might be used for PDT but one has to ensure that the emission spectra of the IPL-applicator must match most suitably to the absorption spectra of Pp-IX, the actual photo-sensitizer in 5-ALA PDT.

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In vivo evidence for the protective role of endogenous urocanic acid against UVB-induced DNA damage in the skin

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Urocanic acid (UCA) is produced by the enzyme histidase and accumulates in the stratum corneum. Exogenously applied UCA has been demonstrated to protect the skin against UV-induced damage, whereas an experimental evaluation of the role of endogenous UCA in photo-protection has been lacking so far. Here we utilized a mouse model, in which the gene encoding histidase is mutated, to investigate the photo-protective role of UCA *in vivo*. Histidase protein was strongly expressed in the stratum granulosum of wild type mice whereas it was undetectable by immunohistochemistry in mutant mice. Accordingly, the concentration of UCA in the stratum corneum of homozygous mutant mice was reduced by approximately 90%.Extracts from tape stripped stratum corneum of mutant mice had less than 10% of the UVB absorption capacity of extracts from normal controls, suggesting that endogenous UCA is indeed the main natural sunscreen of the stratum corneum. When newborn mice and the shaved back skin of adult mice were irradiated with 250 mJ/cm^2 UVB, mutant mice accumulated significantly more DNA damage in the form of cyclobutane pyrimidine dimers (CPDs) than wild type mice. The increase in DNA damage led to a significant increase in the number of apoptotic keratinocytes as determined by labeling of active caspase-3 and of DNA fragments in histidase-mutant mice. Taken together, these results provide strong evidence for an important contribution of endogenous UCA to the protection of the epidermis against the damaging effects of UVB.

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Interaction of linoleic acid and singlet oxygen after UV-A radiation: Does this have an influence on the skin barrier function?

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The use of linoleic acid (LA), an unsaturated omega-6 fatty acid, for the treatment of chronic and acute inflammation of the skin has been shown as very effective to maintain the barrier function of the skin. The discovery of the so-called peroxisome proliferator-activated receptors (PPARs) at the beginning of the last decade of the20th century has opened an interesting new perspective on the use of LA, because LA was shown to be a strong activator of PPARs and exhibited positive effects on the epidermal barrier as well as anti-inflammatory effects *in vivo*. UVA radiation has been known to generate reactive oxygen species such as singlet oxygen, which causes oxidation of lipids and proteins in skin. Therefore we investigated the role of unsaturated fatty acids for UVA-induced generation of singlet oxygen depending on the number and position of double bonds as well as the cellular expression of differentiation and proliferation of keratinocytes *in vitro* via PPAR triggering. Singlet oxygen was directly detected by time resolved measurement at 1270 nm and spectrally resolved from 1150–1400 nm in near-backward direction in respect to the exciting beam using an infrared-sensitive photomultiplier. Different poly-unsaturated LA, c9, t12-LA (isolated double bonds), both conjugated c9, t11-CLA, t10, c12-CLA and a saturated fatty acid (stearic acid) were irradiated with broadband UVA light. The amount of singlet oxygen generation increased with the number of double bonds (0–4). Conjugated c9, t11-CLA and t10, c12-CLA with two adjacent double bonds generates singlet oxygen to a lesser amount (3.5-fold) as compared to the non-conjugated c9, t12-LA. Auto-oxidation processes change the chemical structure of the poly-unsaturated fatty acids during UVA radiation but not the conjugatedc9, t11-CLA and t10, c12-CLA which was confirmed by HPLC. The increase of absorbing UV-A hydro-peroxides leads to an increase of singlet oxygen molecules as shown by an increase of luminescence photons. This process continues as long as hydro-peroxides are present or hydro-peroxides are newly generated by non-oxidized fatty acids. Expression of transglutaminase-1 and involucrin, two key proteins of keratinocytes differentiation, was enhanced significantly after incubation with thec9, t11-CLA and t10, c12-CLA, via binding to PPARs. Overall conjugated LA and UVA radiation seems to contribute to a lesser extent to the production of endogenous singlet oxygen and further oxidative processes as poly-unsaturated fatty acids.

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Antioxidant activities of the flavonoid extract RL-40 in cell-free and cell-based test systems

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The flavonoid extract RL-40 obtained from Reseda luteola L. contains high amounts of flavonoids (40% w/w), mainly luteolin, luteolin derivatives and apigenin (Wölfle et al. 2009). We have previously shown that RL-40 inhibits ultraviolet-B (UVB) induced skin inflammation in a dose-dependent manner *in vivo* (Casetti et al. 2009). Reactive oxygen species play a major role in ultraviolet-induced skin inflammation and photo-aging. Therefore, we were interested in the ultraviolet-absorbing and antioxidant properties of RL-40. Spectro photometric measurements were performed with 1% (v/v) RL-40 and revealed an extinction profile of RL-40 roughly corresponding to that of luteolin, with extinction maxima in the UVB (<280 nm) and UVA (350 nm) range. Ultraviolet transmission below 370 nm was < 10%. The free radical scavenging activity of RL-40 was assessed using cell-free and cell-based assays. In the cell-free 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay the IC50 of RL-40was 26 μg/ml (pure luteolin: 9 μg/ml; Trolox: 32 μg/ml; N-acetylcysteine: 83 μg/ml). In UVB irradiated (60 mJ/cm^2) HaCaT cells the formation of 2, 7-dichlorofluorescein (DCF) by reactive oxygen species (ROS) was reduced by RL-40 in a concentration-dependent manner. RL-40 (IC50 4 μg/ml) was much more effective compared to pure luteolin (IC50 39 μg/ml), Trolox (IC50 161 μg/ml) and N-acetylcysteine (IC50 1100 μg/ml). We conclude that due to its ultraviolet-absorbing and antioxidant properties RL-40 is an interesting active ingredient for sun-protecting and anti-aging topical formulations.

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P219

Steroids and ultraviolet radiation affect contact hypersensitivity differently and by different mechanisms

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Low dose ultraviolet radiation (UV) prevents the induction of contact hyper-sensitivity (CHS) and induces tolerance via induction of regulatory T cells (Treg). We recently-observed that UV-damaged but still viable Langerhans cells (LC) which emigrate from the epidermis into the lymph nodes are required for the induction of Treg. Consequently the steroid mometasone which, in contrast to UV, does not damage but kills LC prevented the induction of CHS like UV, but did not induce Treg. Accordingly, upon steroid application no Langerin-positive cells could be detected in the draining lymph nodes by FACS analysis, suggesting that dendritic cells from the epidermis do not reach the lymph nodes because being killed by the steroid. To confirm the crucial role of LC in the induction of Treg, Langerin-DTR mice were used in which Langerin-positive cells can be depleted by the injection of diphtheria toxin (DT). Langerin-DTR mice injected with DT revealed a lower but still significant CHS response when compared to wild type mice. Langerin-DTR mice not injected with DT were suppressed in their CHS response upon UV like wild type mice. Surprisingly LC-depleted mice revealed a full CHS response upon UV. Accordingly, adoptive transfer experiments revealed that Treg did not develop in UV-exposed LC-depleted mice. The fact that Langerin-depleted mice are resistant to UVR-induced immunosuppression implies that after depletion of Langerin-positive cells other antigen presenting cells (APC) resistant to UV have to present the antigen and that UV-exposed LC might exert the capacity to suppress these cells. When Langerin-DTR mice after injection of DT were treated with mometasone the CHS response was completely suppressed. This implies that, in contrast to UV, steroids kill all APC in the skin resulting in the suppression of the induction of CHS but also in the failure to induce Treg. In contrast upon UV, UV-damaged but still viable LC appear to suppress other APC, thereby preventing induction of CHS. Furthermore, UV-damaged LC migrate into the lymph nodes and there induce Treg. This implies that immunosuppression by steroids is a passive phenomenon due to killing of all APC in the skin, whereas immunosuppression by UV appears to be an active process in which UV-exposed LC may inhibit neighboring APC, migrate into the lymph nodes and there induce Treg.

P220

Human keratinocytes cooperate with fibroblasts in the UVB-induced synthesis of 1α, 25-dihydroxyvitamin D3 (calcitriol) within a three-dimensional cell culture model

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The skin is the only tissue yet known in which the complete UVB-induced pathway from 7-dehydrocholesterol (7-DHC) to hormonally active calcitriol occurs under physiological conditions. The production of calcitriol in the skin strongly depends on the photosynthesis of vitamin D3, which is biologically inactive in the first instance. Vitamin D3 serves as the starting substrate for two subsequent enzymatic hydroxylations in epidermal keratinocytes. By contrast, dermal fibroblasts synthesize only precursors (vitamin D3 and 25-hydroxyvitamin D3 (25OHD3)) of calcitriol *in vitro*. It was the aim of this study to proof whether cooperative effects between keratinocytes and fibroblasts using a specific three-dimensional cell culture model contribute to higher synthesis rates of calcitriol in keratinocytes. We found that fibroblasts grown at the lower surface of a permeable collagen membrane and keratinocytes seeded on the opposite side of this membrane increase the UVB-induced synthesis of calcitriol in keratinocytes supplemented with 1 mM/ cm27-DHC compared with keratinocytes alone. The synthesis rate of calcitriol was positively correlated with the applied dose of UVB radiation (5, 10 and 20 mJ/cm^2) at a wavelength of 300 nm. Surprisingly, we found a low-grade calcitriol synthesis in fibroblasts alone after irradiation with low UVB dose (5 mJ/cm^2) under the same conditions. In conclusion, the cutaneous concentrations of both vitamin D3 and25O HD3 as well as the activity of anabolic and catabolic vitamin D hydroxylases determine the epidermal production of calcitriol, which regulates a number of genes in keratinocytes and other vitamin D receptor (VDR) positive skin cells. It is well known, that both calcitriol and UVB radiation exert potent anti-psoriatic and other beneficial effects in human skin. We hypothesize from our findings, that the anti-psoriatic effect of UVB radiation is attributed at least in part to UVB-triggered cutaneous synthesis of calcitriol most probably due to metabolic cooperation between epidermal keratinocytes and dermal fibroblasts.

P221

Modern tattoos contain azo pigments - identification and quantification of tattoo pigments in human skin specimen

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Tattooing is a very popular body adornment and about 10% of population is tattooed in Germany. The tattoo colorants predominantly consist of black inks or colored azo pigments. The exposure of tattooed skin to solar light for many years may cause a decomposition of especially azo pigments, which has been already shown *in vitro* and ex-vivo. In addition, laser light is applied to destroy the pigments, in case a patient decides to get his tattoo removed by medical laser therapy. Part of these decomposition products are toxic or mutagenic and may pose a risk on tattooed individuals. The risk should be correlated to the amount of pigments that has been decomposed over years inside skin. As a first estimate, this amount is correlated to the difference of the initial concentration of pigments directly after tattooing and the amount of pigments detected years after tattooing. The latter concentration can be determined by extracting the pigment from skin using tattooed skin from forensic medicine (Department of Forensic Medicine, Munich).

The fatty tissue of skin has been removed with a scalpel and the skin has been stored at - 80°C. For identification and quantification of the pigments, we made three punch biopsies with 5 mm in diameter, and extracted the pigments as previously established. The azo pigment in each skin sample was identified and quantified by HPLC DAD technology. We found that about 90% of initial pigment concentration, which is about 2.5mg/cm^2, has disappeared. This is due to pigment decomposition by solar radiation. However, also pigment transportation to other locations inside the human body may play a role. This is an alarming fact since many people have many and large tattoos involving high amounts of pigments that have been punctured in skin. Moreover, most of the people receive their first tattoo in the age of 15 to 20 years and the pigments and the possible hazardous decomposition products may stay in the human body for decades.

P222

Solar UV exposure of people in an urban environment

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In an urban environment most people spend most of their time indoors. Outdoor activities, both in the leisure time and during working hours occupy only a small portion of the daytime. Furthermore, the ambient ultraviolet radiation (UVR) in an urban environment is mitigated by air pollution and the shadowing by buildings. The aim of our study was to find out activities which may contribute significantly to the UV induced health risk in such a low exposure environment. We measured the personal UV exposure as erythemally effective solar UVR during outdoor activities typical for an urban environment from May to August: shopping in a shopping street, walking in a park, sitting in a sidewalk café, cycling in an upright position, sight seeing in open places and spending spare time at an open air swimming pool. Beside the sun burn time (MED divided by the exposure), measured with an optoelectronic personal UV meter (X- 2000, gigahertz, Germany) which was fixed on the chest of one test person, we used the UV index for risk assessments. Generalization of our results was done by calculating ratios of the increase of personal exposure to the actual ambient UVR for all activities. UV exposure was by far the highest when our study subject stayed at the outdoor swimming pool. The sun burn time has a minimum value around 30 minutes for skin type-2. For all other activities except shopping the sun burn time fell into the range up to 1h. During shopping, however, we found no increase of the risk since the sunburn time exceeded several hours. On a time scale with a resolution of 10 minutes the ratio of the effective to the ambient UVR may exceed 1.0 at the swimming pool, whereas during shopping the ratio was below 0.2. For all other activities this ratio was around 0.7. With respect to photo damage we found that at high solar elevation (>45°) photo-protective measures should be applied for certain activities even within a city.

P223

Facial solar UV exposure of farmers during occupation

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Face, neck, lower arms and hands of outdoor workers are chronically exposed to ultraviolet radiation (UVR) during working time whereas all other parts of the body are mostly protected by clothing. This is the reason why the skin aging process is much more pronounced in these areas then in protected regions. We used optoelectronic personal UV meters (X- 2000, gigahertz, Germany) with high temporal resolution to measure the facial solar erythemally effective UV exposure during working time continuously from April to October. The dosimeters possess one UVB- and one UVA-sensitive sensor. With that it is possible to mimic the spectral sensitivity for the human erythema. We applied several quality assurance procedures to ensure high quality measurements such as absolute calibration of each meter with respect to solar elevation and total ozone column. The test persons - 12 full-time farmers - who carried the dosimeters on the forehead fixed to a headband or a cap had to fill in a digital diary on an hourly base in order to assess the UV exposure with respect to the occupational activity. Measurements made obvious that the total cumulative solar exposure of farmers within the 6 months period ranges from 77 SED to 758 SED. This period (April to October), however, contributes with 82% to the annual ambient radiant exposure. Simple extrapolation leads to annual exposures of farmers between 100 SED to1 000 SED per year which is equivalent to an exposure between 3% and 26% of the ambient radiation (horizontally oriented, free horizon). Further on, occupational UV exposure depends on the type of farm, degree of automation of working processes and, most importantly, gender. UV exposure of female farmers was approximately double as high as that of man. During occupation women received 15% of ambient radiation whereas men got only 8%. Our study shows that there is urgent need for photo-protective measures for skin areas regularly exposed to solar radiation. Beside consequent use of sunscreens and broad- brimmed hats workplace measures to prevent situation with very high UV exposures may be helpful.

P224 (V08)

Influence of IL-23 and IL-12 on photo-carcinogenesis

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Ultraviolet radiation (UV)-induced DNA damage is the major basis for the development of UV-mediated skin cancer. Accordingly, reduction of DNA damage lowers the risk for photo-carcinogenesis. The cytokines interleukin (IL)-12 and IL-23have been shown to exert the capacity to reduce UV-induced DNA damage. IL-12and IL-23 are related hetero-dimeric cytokines, consisting of a p35 (IL-12) and a p19chain (IL-23) but sharing the same p40 chain. Both cytokines can be produced in the skin, thus the question is obvious whether endogenous IL-12 and IL-23 protects from photo-carcinogenesis. Previously we could demonstrate that mice deficient in the p40chain have an increased risk to develop UV-induced skin tumors. Since these knock-out mice lack both IL-12 and IL-23 one cannot differentiate the specific role ofIL-12 or IL-23 in photo-carcinogenesis. To clarify this issue, we used knock-out mice lacking the p35 (IL-12p35–/–) or the p19 (IL-23p19–/–) chain. IL-12p35–/–, IL-23p19–/–and wild-type (wt) mice were exposed to an established chronic UV regimen which induces skin tumors within 6 to 12 months. The observation period covered 80weeks. Kaplan-Meier analysis revealed an increased probability of tumor development in IL-23p19–/– compared to wt mice, although the difference was just below the significance level (IL-23p19–/– vs. wt: $P = 0.06$). In contrast, the probability to develop a tumor in IL-12p35–/– was comparable to wt mice (IL-12p35–/– vs. wt: $P = 0.94$). Furthermore, there was a tendency that tumors developing in IL-23p19–/–mice exhibited an increased proliferation rate compared to wt mice, which was not observed for tumors obtained from IL-12p35–/– mice. The number of UV-induced clones of p53-mutant keratinocytes was slightly enhanced in both knock-out mice (IL-23p19–/– >> IL-12p35–/–) in comparison to UV-exposed wt mice. When comparing the current data to the previous observations obtained with p40–/– mice which revealed a statistically significant enhanced risk to develop UV-induced skin tumors, it is obvious that with regard to photo-carcinogenesis a loss of one cytokine can be partially compensated by the other related one. Loss of IL-12 appears to be fully compensated by IL-23, but not vice versa explaining the slightly enhanced carcinogenesis risk in the absence of IL-23. Loss or inhibition of both cytokines, however, dramatically increases the risk of UV-induced skin cancer. This may have impact on the development of future strategies utilizing antibodies or inhibitors ofIL-12 and IL-23, respectively, for the treatment of inflammatory dermatoses.

P225

UV-activated lipids derived from lower plants protect skin cells from chemical and UV stress.

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We have recently shown that the lipids1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (PAPC) can be efficiently activated by UVA-1 to induce the cellular stress response and inhibit inflammation. Since such lipids are promising lead compounds for pharmacologic and cosmetic applications, we searched for novel, botanical sources rich in PUFA. Higher plants cannot produce PUFA, but lower plants, especially mosses are rich sources of these lipids. We hypothesized mosses would contain lipids which could be activated by UVA-1.Using mass spectrometry; we analyzed lipid extracts from *Physcomitrella patens*, and *Sphagnum girgensohnii*, which has been used since the 19th century in wound healing applications. Both mosses contained high levels of PAPC and other PUFA. Accordingly, UVA-1 treatment of the lipids led to their oxygenation. We treated cultured dermal fibroblasts, keratinocytes and HaCaT with native and irradiated lipid extracts from both mosses. Using qPCR and western blot we found that the Nrf2dependent antioxidant response genes were induced by the activated but not the native lipid extracts. When we treated three dimensional skin equivalents with the extracts, we found corresponding regulation. Further, pretreatment of various cell types with activated extracts could inhibit expression of inflammatory cytokines after stimulation with pro-inflammatory agonists. Also UVB-induced cytokine expression was inhibited by the active lipid extracts. These data suggest that these substances have potential for pharmacologic and photo-protective applications.

P226

Enhanced contact allergen and UVB induced keratinocyte apoptosis in the absence of CD95/Fas/Apo-1 from the epidermis

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FAS/ CD95/ Apo- 1 is an ubiquitously expressed cell surface receptor involved in the initiation of programmed cell death. Its function in epidermal keratinocytes has been incompletely defined. Available evidence from *in vivo* studies points to important roles of Fas in the pathogenesis of contact dermatitis and in keratinocyte apoptosis induced by ultraviolet light. To define functions of Fas in the epidermis *in vivo*, we have generated mice with epidermis specific deletion of Fas and have tested its requirement for DNFB induced contact dermatitis and for UVB induced keratinocyte apoptosis. Using a genetic *in vivo* model we show here that expression of Fas by epidermal keratinocytes is neither necessary for the normal development of contact hypersensitivity of the skin, nor required for keratinocyte apoptosis following UVB irradiation. Surprisingly, both contact hypersensitivity and UVB induced keratinocyte apoptosis was significantly enhanced in Fas negative epidermis. Our results thus demonstrate that, in the epidermis *in vivo*, Fas exerts anti apoptotic functions that outweigh its pro apoptotic functions in contact hypersensitivity responses of the skin and in the tissue response of the epidermis to UVB irradiation. These new insights into the function of Fas in the epidermis will have influence on the therapeutic approach to skin conditions characterized by increased keratinocyte apoptosis, such as drug reactions and sun burn.

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UVB radiation induces skin hemorrhage during thrombocytopenia

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Platelets have been recognized as important mediators of vascular integrity during inflammation and tumor angiogenesis, the precise mechanism being matter of ongoing research. In the present study we investigate the role of platelets for vascular stability and prevention of skin bleeding in thrombocytopenic mice. Exposure of mice to UVB radiation shows a dose-dependent induction of skin hemorrhage. We observe that hemorrhage is strictly limited to the sites of UVB stimulation however the cellular and molecular mechanisms of this finding have not been elucidated yet. Using histology and biochemical skin analysis (MPO, Hb, Evans blue leakage) we investigate the role of innate immune cells in the induction of skin hemorrhage. By means of *in vivo* imaging in the dorsal skin fold chamber we further follow the cellular steps inducing skin bleeding. Our findings reveal that for prevention of skin hemorrhage UV-protection is essential during thrombocytopenia.

P228 (V15)

Cellular inhibitor of apoptosis proteins (cIAPs), but not XIAP inhibit a crypticCD95-induced cell death by limiting RIP1 kinase recruitment to the death receptor complex (DISC)

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Inhibitor of apoptosis proteins (IAPs) such as cellular IAP1 or IAP2 (cIAPs) or X-linked IAP (XIAP) are important regulators of cell death pathways with relevance during tumorigenesis. Consequently cIAPs or XIAP may represent promising therapeutic tumor targets. Over the past years chemical IAP antagonists have been developed that mimic binding of the natural XIAP antagonist smac/DIABLO to XIAP. In this report we have investigated the impact of IAP antagonists in keratinocytes and squamous cell carcinoma (SCC) cells of the skin in respect to their cell death-regulatory potential. Whereas XIAP has been demonstrated to represent apotent caspase inhibitor, the function of cIAPs has been more obscure, and mechanistic explanation of their role in cell death pathways is lacking to date. Here we demonstrate that the loss of cIAPs (using shRNA expression of cIAP1 or 2or an IAP antagonist able to degrade cIAPs within minutes) leads to a dramatic sensitization to CD95L or TRAIL killing in skin tumor cells. Surprisingly, two different forms of cell death can be observed in the absence of cIAPs: we observed not only canonical caspase-dependent cell death, but also a second form of caspase-independent cell death that is unmasked in the absence of cIAPs. This form of cell death is dependent on the kinase activity of RIP1 kinase. Consequently knockdown (by shRNA expression) or knockout of RIP1 (as studied by RIP1knockout mouse embryonic fibroblasts (MEFs)) protected cells from CD95L/IA-Pantagonist induced death. Consistent with these data, we detect a large increase of RIP1 in the CD95 DISC and in a secondary cytoplasmic complex (complex II) in the presence of IAP antagonists. Most surprisingly, only the long isoform of cFLIP, cFLIPL, but not cFLIPS interfered with RIP1 recruitment to the DISC and complex II formation and protected cells from necrotic cell death. Our results highlight the fundamental importance of functional cIAP-RIP1 interaction for caspase-independent death ligand-mediated cell death. Our results support the concept that IAP antagonists may represent promising candidates for tumor therapy of skin tumors, most likely in combination with death receptor agonists currently developed in phase II clinical trials. Importantly IAP antagonists may also allow for the modulation of anti-tumor immune responses due to increased non-apoptotic (inflammatory) cell death upon death receptor ligation.

P229

The farnesyl transferase inhibitor Lonafarnib sensitizes melanoma cells to the multikinase inhibitor Sorafenib by inhibiting Rheb farnesylation and mTOR signaling

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Farnesyl transferase inhibitors (FTIs) inhibit the farnesylation of a number of target proteins, including RAS and RHEB. RAS signals to the RAF-MEK-ERK (MAPK) andPI3K-AKT-mTOR (AKT) signaling pathways which are constitutively activated in melanoma and appear to play a major role in tumor progression and drug resistance. RHEB positively regulates mTOR signaling. We investigated the effects of the FTI lonafarnib alone and in combination with pharmacological MAPK pathway inhibitors or AKT pathway inhibitors on proliferation, survival and invasive tumor growth of metastatic melanoma cells. Lonafarnib alone did not sufficiently inhibit melanoma cell growth, and combinations of lonafarnib with AKT pathway inhibitors did not significantly increase melanoma cell growth inhibition. Combinations of lonafarnib with MAPK pathway inhibitors yielded additional growth inhibiting effects. In particular, the combination of the FTI lonafarnib with the multikinase inhibitor sorafenib synergistically inhibited melanoma cell growth, potently enhanced sorafenib-induced melanoma cell apoptosis and completely suppressed invasive tumor growth of melanoma cells in monolayer and organotypic culture. Apoptosis induction was associated with down-regulation of the antiapoptotic proteins Bcl-2 and in particular Mcl-1 and was abrogated by caspase inhibition. Lonafarnib did not affect MAPK and AKT signaling but mTOR signaling by inhibition of RHEB farnesylation. Together, these findings argue that the FTI lonafarnib inhibits RHEB farnesylation and mTOR signaling and potentiates sorafenib-induced apoptosis in melanoma cells. Apoptosis induction by lonafarnib and sorafenib may be, at least in part due to down-regulation of the antiapoptotic Bcl-2 family protein Mcl-1.

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HLA class I and class II antigen expression in malignant fibrous histiocytoma, fibrosarcoma and dermatofibrosarcoma protuberans is significantly down-regulated

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Abnormality in the expression of major histocompatibility complex (HLA) antigens by tumor cells impairs cellular immune responses promoting immune evasion and tumor survival. To date, studies analyzing HLA class I and class II expression levels in malignant fibrous histiocytomas, fibrosarcomas and dermatofibro sarcoma protuberans are limited. Therefore, we investigated the *in vivo* expression profile ofHLA class I and class II antigens in 99 malignant fibrous histiocytomas (MFH), 20fibrosarcomas (FS) and 34 dermatofibrosarcoma protuberans (DFSP) from different anatomical sites. Immunohistochemistry using monoclonal antibodies to HLA class I and II antigens were used to define the expression levels of these antigens on respective tumor samples. Frequent loss or down-regulation of HLA class I and II expression in malignant fibrous tumors was observed for the different types of tumors examined. The data presented suggests that a high frequency of HLA class I and II abnormalities are present in malignant fibrous histiocytomas, fibrosarcomas and dermatofibrosarcoma protuberans *in vivo*. This information can be used in the practical and clinical design of tumor vaccination strategies and provides information regarding the nature of tumor growth and development.

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Polo-like kinase 1: a potential therapeutic target in human melanoma

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Melanoma is a very rapidly growing and lethal cancer. Disruption of the cell cycle regulation has been implicated in the development and progression of malignant melanoma albeit the exact underlying factors and mechanisms are not characterized yet. By using cDNA microarray technique (to evaluate gene expression in melanocytic nevi, primary melanomas, melanoma metastases and human melanoma cell lines) and by performing pathway enrichment analysis we could identify cell cycle pathway and its member polo-like kinase 1 (Plk-1, a mitotic serine/threonine kinase) but not Plk-2, 3 or 4 to be significantly over expressed in primary melanomas and melanoma metastases. This finding could be confirmed using real-time RT-PCR analysis on an independent set of specimens. In *in vivo* analysis of 8 human melanoma cell lines (including theNCI-60 panel) we observed the peak expression of the Plk-1 to be at the G2/M phase of the cell cycle. To explore the role of Plk-1 in human melanoma cell biology, melanoma cell lines were transfected with commercially available/validated Plk-1siRNA or a pDNA vector transcribing Plk-1 siRNA (constructed in our lab). Both strategies as compared to scrambled siRNA/control pDNA vector led to: (i) significant reduction of Plk-1 mRNA and protein accompanied by significant decrease in cell proliferation, (ii) induction of mitotic catastrophe, (iii) cell death with induction of apoptosis. Plk-1 inhibition can induce apoptosis by a p53-dependent mechanism. However inhuman melanoma cell lines, there was no change in the expression of the master tumor suppressor protein p53. Concomitant siRNA silencing of p53 was not able to rescue the cells further excluding its possible involvement in induction of apoptosis in our system. Further analysis of apoptosis revealed caspase 3/8 dependency and activation of the extrinsic pathway of apoptosis through cleavage of Bid and decrease in Bcl-2. This was followed by the release of mitochondrial cytochrome c at the later time points. There was no change in the expression of the apoptosis inducing factor (AIF), Bak, Bax, Apaf-1 and HIF-1α arguing against the involvement of the intrinsic and for the extrinsic pathway of apoptosis. As comparative genomic hybridization (CGH) and SNP arrays showed no genetic alteration in locus 16p12.1 expressing Plk-1 in our samples, we were interested in possible alternative mechanisms of Plk-1 activation in melanoma. Here, we presume MAPK signaling pathway to induce Plk-1 expression in primary and metastatic melanomas. This pathway is also significantly activated in human melanoma and inhibition of this pathway using the MEK inhibitor P89059 resulted in decreased expression of Plk-1 in human melanoma cell lines. This study shows that: (i) Plk-1 expression is dynamically regulated during the cell cycle of human melanoma and, (ii) knock down of Plk-1 can lead to inhibition of human melanoma cell proliferation, survival and induction of apoptosis. We conclude that Plk-1 could be a potentially attractive target in melanoma therapy, particularly interesting because several small molecule inhibitors of Plk-1 (e.g.: BI2536, GSK461364) are already in pre-clinical and phase I clinical trials.

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The RANK-RANKL pathway plays a role in metastasis of skin tumors

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Skin cancer constitutes one of the most frequent types of malignancies and the RANK-RANKL signaling pathway is critically involved in the migration of tumor cells. Since UV irradiation is an essential risk factor for the development of skin tumors and up-regulates RANKL expression, we have investigated whether RANK and RANKL are expressed on human melanoma, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) cells. Interestingly, flow cytometry as well as quantitative real time PCR revealed a minor expression of RANK and RANKL on melanoma cells from stage I and stage II melanoma patients. In contrast, the RANK and RANKL expression was significantly up-regulated in stage IV melanoma patients having multiple metastases in different organs. Of note, neither RANK nor RANKL were detectable in cancer cells from BCC or SCC patients usually characterized by a reduced tendency to migrate and metastasize suggesting that RANK and RANKL might be up-regulated in migrating tumor cells. To test this hypothesis in more detail we performed immunohistological staining of primary melanomas as well as lymph node metastases. Strikingly, RANK and RANKL expressing tumor cells were detectable in primary melanomas of stage IV melanoma patients as well as in lymph node metastases whereas RANK and RANKL were not expressed in tumor cells of stage I or stage II primary melanomas indicating that the expression of RANK and RANKL might be associated with the metastasis of melanoma cells. Since RANK and RANKL expression in tumor cells has been implicated in tissue-specific metastatic behavior of cancer cells and directed migration towards the bones we analyzed whether in melanoma patients the level of RANK and RANKL expression on tumor cells correlates with the presence or absence of bone metastases. In contrast to breast cancer we did not detect an up-regulation of RANK or RANKL in melanoma patients with bone metastases compared to stage IV patients without bone metastases. Together, these data demonstrate that RANK and RANKL are expressed on melanoma cells from stage IV patients suggesting that the RANK-RANKL signaling pathway might be involved in the migration of melanoma cells and melanoma metastasis formation.

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Dual targeting of RAF and mTOR potently induces apoptosis in melanoma cells

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In melanoma, the RAF-MEK-ERK (MAPK) and the PI3K-AKT-mTOR (AKT) signaling pathways are constitutively activated and have key functions in tumor progression. However, clinical studies revealed that neither the multikinase/RAF inhibitor sorafenib nor the mTOR inhibitor sirolimus have sufficient antitumor activity in patients with metastatic melanoma. Recent experimental data suggest that in melanoma an effective treatment strategy must take into account more than one de-regulated signaling pathway. We investigated whether combined targeting of both pathways with the RAF inhibitors or a fenib and the mTOR inhibitor sirolimus has therapeutic effects in melanoma. The effects on growth, survival and invasion of six human metastatic melanoma cell lines in monolayer and organotypic culture were investigated. Combination of sorafenib with sirolimus significantly potentiated growth inhibition in all melanoma cell lines tested and led to an approximately 2-fold increase of apoptosis compared with sorafenib mono treatment ($P < 0.05$). These effects were associated with down-regulation of the antiapoptotic proteins Bcl-2 and Mcl-1. Moreover, sorafenib in combination with sirolimus completely suppressed invasive melanoma growth in organotypic culture. To investigate the molecular mechanisms involved in the antitumoral action of this drug combination we analyzed the gene expression profile of melanoma cells in response to sorafenib and sirolimus. Data analysis showed a series of stress-associated genes that were up-regulated after combination treatment with sorafenib and sirolimus. One of these genes was that encoding p8, a protein that belongs to the family of HMG-I/Y transcription factors and that is involved in the control of cell fate. Using real-time quantitative PCR, we confirmed that sorafenib and sirolimus up-regulates p8 mRNA levels in all six melanoma cell lines tested. Likewise, western blot analysis showed that sorafenib and sirolimus increases p8 protein levels in melanoma cells. Further analysis of the gene expression profile has led to the identification of three genes encoding the transcription factors ATF3 and CHOP and the stress-related pseudokinase TRB3 which have been proposed to be responsible for the execution of endoplasmic reticulum stress-induced apoptosis. Using real-time quantitative PCR, we confirmed that sorafenib and sirolimus up-regulates ATF3 andTRB3 mRNA levels in melanoma cells. These data suggest that sorafenib in combination with sirolimus potently induces apoptosis of melanoma cells through the endoplasmic reticulum stress pathway.

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Investigation of all-trans-retinol oxidation in malignant and benign skin cells

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Vitamin A serves as substrate for the biosynthesis of several derivates (retinoid) which are important for cell growth and cell differentiation as well as for vision. All-trans-retinoic acid (ATRA) is the major physiologically active form of vitamin A regulating the expression of different genes. At present, hundreds of genes are known to be regulated by retinoic acid. The biosynthesis of ATRA includes the oxidation of all-trans-retinol (ATRol) through all-trans-retinal (ATRal) as an intermediate product. For ATRol oxidation a number of cytoplasmic and microsomal alcohol dehydrogenases (ADHs) are responsible. In melanoma cell lines, melanocytes and keratinocytes we found no oxidation of ATRol in ATRA. One possible explanation of this phenomenon could be an up-regulation of ADHs expression or a disturbance in their enzymatic activity. First, we analyzed an activity of cytosolic NAD+-dependent ADHs (ADH1, ADH2 and ADH7), a microsomal NAD+-dependent ADH (RoDH4) and a microsomal NADP+-dependent ADH (RoDH1) in melanoma cell lines and in a keratinocyte cell line HaCaT. We found about a 5-fold increase in the cytosolic NAD+-dependent ADH activity in melanoma cells compared to keratinocytes. In contrast, the RoDH4 activity was higher in the HaCat cell line than in melanoma cell lines. The RoDH4 activity was very low in all melanoma cell lines (except for Ma-Mel-04) and in HaCaT. An analysis of ADHs expression revealed the absence of ADH1, ADH2, ADH4 and RoDH4 expressioneither in malignant (melanoma) and in normal skin cells (melanocytes and keratinocytes). Expression of ADH7 was found only in melanocytes and keratinocytes. All melanoma cell lines, melanocytes and keratinocytes were capable of expressing RoDH1. Stimulation of melanoma cell lines with retinoids led to an increase of RoDH1 activity. Thus, both malignant and benign skin cells express constitutive RoDH1 activity, but the activity is too low to oxidize the ATRol.

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Vitamin A metabolism in melanoma cells vs. melanocytes: Importance of lecithin/retinol acyl transferase and RPE65

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Vitamin A (all-trans retinol, ATRol) and its biological active metabolites - retinoids -such as all-trans retinoic acid (ATRA) are essential molecules that are able to influence cell growth, differentiation and apoptosis by controlling various signaling pathways. For this reason, retinoids are also regarded to be important in the context of tumor development and progression. Accordingly, an aberrant vitamin A metabolism could be detected in several epithelial tumors leading to dysfunctional gene regulation mechanisms. Essential enzymes in vitamin A metabolism are alcohol dehydrogenases (ADHs), which catalyse all-trans retinal (ATRal)/ATRol conversions, the ATRal/ATRA-converting aldehyde dehydrogenases (ALDHs) as well as lecithin/retinol acyl transferase (LRAT) and RPE65, which are important for the esterification of ATRol. In this work, we present melanoma specific alterations in the expression of these enzymes and reveal their consequences for retinoid metabolism in melanoma by activity measurements. The mRNA expression of different ADH and ALDH enzymes in melanoma cells and melanocytes are similar, but melanoma cells and melanocytes show different general ADH and ALDH activities. In contrast to melanocytes which express no LRAT and RPE65 at the protein level, LRAT andRPE65 are present and functional in melanoma cells. Hence, we could show that melanoma cells prefer the reduction of ATRal with the subsequent esterification of ATRol by LRAT, while melanocytes favor only the reduction of ATRal to ATRol. Whereas the treatment of melanoma cells with ATRol, ATRal and ATRA does not induce a uniform expression pattern of these genes, this retinoid treatment leads toup-regulation of LRAT mRNA expression in melanocytes, suggesting that ATRol and ATRal are able to modulate gene expression such as ATRA. Therefore, we propose that the removal of ATRol in melanoma cells by LRAT and RPE65 leads to a disturbance in cellular retinoid level. Consequently, the decreasing cellular amount of ATRA and its precursor molecules should result in a change of gene regulation.

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Platelets interact with B16 melanoma cells to augment tumor cell adhesion and promote lung metastasis formation

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Recently, fundamental aspects determining the molecular basis for the reciprocal relationship between metastasizing tumor cells and soluble components of the coagulation cascade have increasingly become understood. However, the involvement of platelets, the cellular component of thrombus formation, in the pathogenesis of cancer metastasis is still poorly comprehended. Here, we demonstrate that platelets are an important contributing factor in the early phase of the formation of lung metastasis *in vivo*. As determined by *in vivo* luciferase bioluminescence imaging and *in vivo* bioluminescence analysis of lung tissue, the depletion of platelets after i.v. inoculation of luciferase-transduced murine B16melanoma cells (B16M-luc) into syngeneic C57BL/6 mice resulted in a more than 30% decrease in micrometastasis to the lung. Thus, we hypothesized that platelets maybe critically involved in the multistep process of tumor metastasis. Evaluating tumor cell rolling (parallel-plate flow chamber), chemotaxis (wound assay) and migration (transwell chamber) we found no significant platelet mediated effect on B16Mbehavior *in vivo*. However, we determined an up to 50 fold augmentation in the adhesion of melanoma cells on immobilized platelets under static conditions. Furthermore, platelets substantially mediated an up to 6fold increase in B16melanoma cell (B16M) adhesion to endothelial cells under physiological shear stress conditions. The observed increase in tumor cell adhesion was significantly abolished by blocking monoclonal antibodies (mAb) to alpha V integrin, an adhesion molecule highly expressed on 97.8% of B16M. Likewise, blocking mAb to GPIIbIIIa, the most abundant platelet adhesion receptor, and the integrin blocking peptide RGD both significantly abrogated the observed platelet-mediated increase in B16M adhesion. We conclude that platelet-tumor interactions are critically involved in the early formation of metastasis of melanoma cells to the lung and suggest that the specific targeting of adhesion molecules involved in this process may represent a promising strategy for therapeutic intervention.

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Neonatal UV irradiation promotes multistage melanomagenesis in theHgf-Cdk4R24C C57BL/6 mouse tumor models

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Intermittent UVB exposure during childhood is a major risk factor for the development of melanoma. Genetically engineered mouse models offer new opportunities to investigate the role of UVB radiation in melanoma pathogenesis. We establishedC57BL/6 mice where aberrant growth factor signaling due to transgenic overexpression of the hepatocyte growth factor (Hgf) and impaired cell cycle control due to an oncogenic germline mutation in the cyclin dependent kinase 4 (Cdk4R24C) cooperate to selectively promote stepwise malignant transformation of melanocytes in the skin. All animals in a cohort of 33 untreated Hgf-Cdk4R24C mice developed multiple melanocytic nevi, sporadic primary melanomas and metastases in draining lymph nodes and internal organs including lungs and liver. Importantly, tumors of other histology were only rarely observed. Irradiation of newborn Hgf-Cdk4R24Cmice with a single erythemogenic dose of UVB significantly accelerated multi stage melanomagenesis. The mean age at tumor onset decreased from 272±65 to 192±43 days. UV-induced melanomas could not be distinguished from spontaneous melanomas by histomorphology or by gene expression analyses. They grow progressively and metastasized in a similar fashion. This experimental system is ideally suited to further elucidate the principles how environmental UVB radiation and the individual genetic constitution contribute to melanomagenesis.

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Primary cutaneous melanomas in genetically engineered mice imitate the biology of nodular pigmented melanoma without lymphocytic infiltration in man

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De-regulated receptor tyrosine kinase signaling and impaired cell cycle controls are key features of melanoma in man. To mimic the molecular pathogenesis of the human disease in an experimental model we established a genetically engineered strain of mice with transgenic overexpression of the hepatocyte growth factor (Hgf) and an oncogenic germline mutation in the cyclin dependent kinase 4 (Cdk4R24C).These pathogenetic changes cooperate to selectively promote sporadic malignant transformation of melanocytes in the skin with near complete penetrance. PrimaryHgf-Cdk4R24C melanomas show very little inflammation-associated immune cell recruitment indicating that they effectively evade immune defense during tumor progression. Neoplastic cells spread metastatically to the draining lymph nodes where they diffusely infiltrate the T cell-rich areas and do not activate dendritic antigen-presenting cells. Quantitative RT-PCR analyses demonstrate high expression levels of marker genes characteristic for progressively growing melanoma but very low expression levels of pro-inflammatory cytokines and chemokines. Histomorphologically, Hgf-Cdk4R24C melanomas in mice resemble a subset of pigmented nodular melanomas in man which grow invasively without lymphocyticin filtration and frequently metastasize to the sentinel lymph nodes. A detailed analysis of primary tumors and sentinel lymph node biopsies for 85 melanoma patients substantiates the notion that the Hgf-Cdk4R24C mouse melanoma model imitates the biology of this subset of patients with a poor prognosis.

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Tight Junction-specific proteins are absent from melanoma while ZO-1 is widely expressed

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Melanoma (MM) is the most aggressive form of skin cancer and is highly resistant to conventional chemotherapy, immunotherapy and targeted therapy. In recent publications the expression of TJ proteins in MM tissue and cultured MM cells was described on RNA and on protein level. The presence of these proteins would have two important impacts on the molecular understanding and treatment of MM: First, these proteins might form functional TJ which could result in the encapsulation of specific areas of the tumor which might be no longer accessable for the immune system or for therapeutics. These TJ would have to be specifically opened for successful therapy. Second, TJ proteins might be used as targets for tumor therapy. In order to investigate the attendance of TJ proteins in MM in more detail we examined 64 cases of MM, 59 cases of nevi, 6 MM cell lines and melanocyte primary cultures for the expression and localization of various TJ proteins. First results seemed to confirm the described findings concerning the presence of Cldn-1 and additionally revealed the presence of JAM-A in MM. However, more detailed investigations showed that Cldn-1 positive cells were epithelial cells present in some areas of the tumors. JAM-A positive cells were proven to be CD43 positive cells. All tumors were negative for Occl and Cldn-4. Only ZO-1 was clearly positive in MM cells, but this protein can also associate with adherens- (AJ) and gap-junctions. Also cultured MM cells as well as melanocytes were negative for Cldn-1 and Cldn-4and only one MM cell line was positive for occludin. All lines were positive for ZO-1and JAM-A, however, the latter was only found in the cytoplasm. Pull down assays revealed an interaction of ZO-1 and N-Cadherin in MM cells, demonstrating its association with AJ but not TJ in these cells. As in a previous study a knock down of ZO-1 in MM cells resulted in reduced invasion of the cells into collagen gels we investigated the detailed localization of this protein in MM. Interestingly, 53% of MM showed increased staining intensity in invasive areas while this was not the case in any of the nevi. Correlation to metastasis and survival are current under examination. Summarizing our results we could not detect any TJ-specific proteins in MM. Therefore we conclude that MM does not build up functional TJ which reduce the accessibility for therapeutics. ZO-1 is likely to be associated with AJ proteins and might be involved in invasiveness of tumor cells.

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Investigation of the role of Merkel cell polyomavirus (MCPyV) in Merkel cell carcinoma cell lines (MCCL).

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Merkel cell carcinoma (MCC) is a rare cancer of the skin with neuroendocrine origin. For years an infectious etiology was suggested based on the presence of MCC predominantly in elderly (>70 years) and immunosupressed patients SOT-patients; HIV-patients). Furthermore spontaneous regression after reconstitution of the immune status was reported. Recently, a novel human polyomavirus (MCPyV) was identified in MCC tissue by high throughput transcriptome analysis. High prevalence of MCPyV in MCC tumors (70–85%) and monoclonal integration of the virus in the tumor cells strongly suggest a role of MCPyV in MCC pathogenesis. In this study we analyzed seven MCC cell lines (MCCL) for the presence and copy number of MCPyV. We correlated MCPyV status with MCCL subtype, growth rates, p53 and pRb expression levels as well as collagen-invasion of the cell lines. Five of seven MCCL were positive for MCPyV, but copy numbers of the viral DNA relative to GAPDH varied. Three cell lines exhibited 5-10 viral genome copies per cell while in two cell lines the relative amount of viral DNA was only one copy per 50 cells or less. We did not observe a correlation of the presence of MCPyV with the conventional classification of the cell lines which distinguishes MCCL by expression of neuroendocrine markers and morphological characteristics. In addition, there was no correlation between proliferation of the cell lines and presence and absence of the virus. The same was true for the invasiveness of the MCC cell lines. Interestingly, no correlation of p53 or pRb protein expression levels and the MCPyV presence could be observed, confirming recent data from MCC primary tumors. These results suggest that MCPyV does not influence growth, invasion and protein expression of p53 and pRb in MCCL. One of the most exciting and challenging questions will be to elucidate of the mechanisms of MCPyV in MCC pathogenesis.

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Analysing tumor suppression in malignant melanoma. A systems biology approach

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Development and progression of malignant tumors is due to deregulated cell growth and cell cycle, involving a plethora of different molecules. Among these, tumor suppressor proteins like p53 and p16 play a crucial role. p53 induces 14–3–3σ, a multifunctional inhibitor of intracellular signaling and cell cycle. In addition, p53together with MDM2 and 14–3–3σ form an important intracellular signaling module for growth control, in which 14–3–3σ positively regulates the activity of p53 through feedback mechanisms. To better understand the complex process of tumor suppression in malignant melanoma, we established a mathematical model integrating the effects of 14–3–3σ gene silencing, the dynamics of 14–3–3σ induction and intracellular compartmentalisation and the role of interacting molecules such as p53 and MDM2. *In vivo* experiments with different melanoma cell lines were performed as a basis for our mathematical model and the model was subjected to computer simulations to analyse different scenarios of protein activation and interaction. Our analyses show that strong stimulations are necessary to induce 14–3–3σ expression even in cases of intermediate levels of gene methylation. More important, our model suggests that epigenetic silencing of 14–3–3σ is a major factor affecting p53 dynamics and activity. We found that down-regulation of p53 expression via 14–3–3σ gene methylation and reduction of its nuclear localisation drastically affect its activity. Taken together, the complex regulation of tumor suppression in malignant melanomais described by a mathematical models using a systems biology approach. The proposed model allowed us to predict tumor cell behavior under different conditionsof stimulation and emphasized the important role of gene methylation for tumor malignancy. These findings might open interesting perspectives for future treatment strategies of malignant melanoma and other malignant tumors.

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Maspin expression is related to prognostic parameters and clinical outcome in malignant melanoma

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Background: Maspin was originally considered to be a tumor suppressor in breast and prostate cancer. However, the expression pattern of maspin differs among different cancer types and normal tissue, its significance as a tumor suppressor has been questioned and the expression of maspin in different histopathological subtypes of malignant melanoma (MM) has not yet been systematically examined.

Objective: To investigate the immunohistochemical expression pattern and intensity of maspin staining in different histopathological subtypes of MM and to correlate staining pattern and staining intensity with histopathological criterias and clinical outcome.

Methods: 47 formalin-fixed, paraffin-embedded malignant melanomas (nodular melanoma, $n = 16$; superficial spreading melanoma, $n = 23$; lentigo maligna melanoma, $n = 4$; acrolentiginous melanoma, $n = 3$) were immuno stained with maspin antibodies. Breslow thickness and Clark level of the primary tumor, clinical stage at time of diagnosis, S100 serums levels, progression-free survival (PFS) and overall survival (OAS) were collected for statistical analysis.

Results: Immunohistochemical analysis revealed cytoplasmic expression of maspin in melanoma cells but not in melanocytes from the surrounding normal-appearing skin. Maspin expression was exceedingly high in epidermal keratinocytes, showing no difference in maturation of keratinocytes. Maspin expression differed in the different melanoma subtypes investigated. Maspin expression correlated with prognostic parameters such as Clark level, Breslow thickness and stage of disease.

Conclusion: We could show that maspin expression in MM was associated with the underlying histopathological subtype of melanoma. Strong expression of maspin in MM was associated with poor prognosis of the patient, indicating that maspin may play the role of a tumor promoter in MM and may serve as a prognostic parameter.

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Loss of EGFR function impairs papilloma growth during multi-stage chemical carcinogenesis in the mouse

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Analysis of mice carrying spontaneous and targeted mutations of the epidermal growth factor receptor gene (Egfr) clearly showed that this tyrosine kinase receptor is essential for the proper development and homeostasis of the epidermis and hair follicle. However, since mice lacking EGFR die during embryonic development or during the first weeks of postnatal life, their shortened life span and growth retardation precluded more detailed studies and forced researchers to fall back on graft experiments. Nevertheless, these studies indicate that the EGFR acts as a survival factor for skin tumors cells. Here, we employed the ENU-induced mutant mouse line Waved-5 (Wa5) to analyze the impact of significantly reduced EGFR signaling during multi-stage chemical carcinogenesis. Wa5 mice have a point mutation resulting in an antimorphic Egfr allele whose product acts as a dominant negative receptor, strongly inhibiting the wild-type EGFR. Seven-week-old Wa5females and control littermates received a single application of the initiating agent7,12-dimethylbenz(a)anthracene (DBMA) followed by multiple applications of the promoting agent 12-O-tetradecanoylphorbol-13-acetate for 26 weeks. We found thatWa5 mice remained free of papillomas for a longer time than control littermates. Only50% of Wa5 mice showed at least one tumor 20 weeks after DMBA treatment while100% of control mice had at least one tumor at this time point. In addition, Wa5 mice developed significantly fewer tumors. In contrast, the mean tumor size was not different between Wa5 and control mice. The present data indicates that EGFR signaling contributes to tumor growth during multi-stage chemical carcinogenesis of the skin in mice.

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Role of microRNAs in melanoma metastasis

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The posttranscriptional regulation of gene expression by small, non-coding RNAs, so-called microRNAs (miRNAs), was first described in the early nineties of the last century. miRNAs specifically bind to the 3'-untranslated region (3'-UTR) of an mRNA and lead to either degradation of target mRNA or to inhibition of mRNA translation. In large-scale analyses of breast, colon, and lung cancer, different forms of leukaemia and malignant melanoma, tumors showed significantly different miRNAs patterns compared with benign tissues, suggestive for a functional role of miRNAs in tumor development. Indeed, functional experiments showed that miRNAs may significantly impact on tissue invasion, cell cycle regulation and cell growth of a variety of tumors. Little is, however, known about the role of miRNAs in tumor progression and metastasis. Here we analysed the expression of a panel of 157 different miRNAs in primary melanomas ($n = 10$) and cutaneous melanoma metastases ($n = 20$) using laser-microdissected tumor tissues. We also put a focus on miRNA target genes with more than four binding sites for different miRNAs in their 3'-UTR, because these so-called target hubs had been shown to be of central importance for intracellular protein networks. Among the top differentially expressed miRNAs were miR-222, miR-21 and let-7a, which were significantly up-regulated in metastases compared with primary tumors. miR-222 targets p27(Kip1), a well-known tumor suppressor gene, which has been shown to be down-regulated during melanoma progression in earlier immunohistochemical studies. In functional experiments, we were able to demonstrate that transfection of miR-222 dramatically down-regulated p27 (Kip1) in a series of melanoma cell lines and reduced reporter gene activity of a p27 3'-UTRconstruct. These experiments are highly suggestive for a direct functional interaction between miR-222 and p27 (Kip1) in melanoma. In further experiments addressing the identification of regulatory intracellular protein networks, more than 10 candidate genes were identified as putative target hubs. Among these were sequences with as yet unknown function but also members of the Ras family of intracellular signal transducers and genes involved in glucose metabolism. Taken together, our analyses identified miRNAs which might explain impaired tumor suppression in late-stage malignant melanoma and candidate genes of protein networks with a putative role during melanoma progression. The latter might serve as targets for future treatment strategies.

P245

Unexpected induction of delayed type hypersensitivity (DTH) to human serum albumin in melanoma patients by Dendritic Cell vaccination

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Tumor antigen-loaded dendritic cells (DC) are experimental cancer vaccines which induce immunity and in a subset of patients also clinical effects without severe side effects. IRAEs (immune related adverse events) like inflammatory responses localized to the injection site, rise of temperature and fatigue usually occur, however, as soon as immunity is induced (often at the 2nd or 3rd vaccination) with a delay of hours to a few days. DTH reactions mediated by T cell infiltrates at the vaccination site are frequent and indicate immune induction to vaccine antigens. Generalized dermatitis-like eruptions are only occasionally observed and are often interpreted as induction of auto-immunological processes. Here we describe the occurrence of extensive local and generalized reactions following vaccination with DC loaded with multiple classes I and II peptides. In 5 out of49 patients treated a generalized maculopapular exanthema occurred concurrently with particularly extensive local reactions. We established that the DC vaccine, which had been cryopreserved in pharmaceutical quality Human Serum Albumin (HSA), induced specific T cell reactivity against the HSA protein rather than some contaminants. Intracutaneous skin test results concurred with immunological *in vivo* assays in that a DTH reaction occurred, while the absence of IgE antibodies as shown by Western Blot was in accordance with negative prick tests. In select patients it was shown that upon switching to DC frozen without HSA a rash no longer occurred but resurged when the HSA-containing vaccine was used again. This is the first report that a vaccine induces T cell immunity to HSA protein which is a self-protein. This finding point to the unique immunogenicity of DC vaccines which should, therefore, be cryopreserved in the absence of commercial HSA which may be, for example, contain aggregates resulting in altered antigen processing and formation of neo-epitopes.

P246 (V10)

Differential regulation of cadherins through Slug and Twist in melanoma

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Epithelial-mesenchymal transition (EMT) is a process which is crucial for initiation, development and metastasis of epithelial tumors. One hallmark of EMT is the repression of the epithelial cell adhesion molecule E-cadherin and the up regulation of the mesenchymal marker N-cadherin. This switch enables melanocytic cells to interact with N-cadherin expressing fibroblasts and thus promotes tumor invasion and migration. Here we demonstrate that changes in the expression levels of Slug and Twist, two epithelial-mesenchymal transcriptional regulators (EMTRs), exert differential effects on the overall expression and adhesive as well as migratory properties of melanoma cells. Slug and Twist were down regulated using siRNA in the metastatic melanoma cell line WM164, a cell line expressing Slug and Twist as well as E- and N-cadherin. Overexpression of Slug in metastatic cell lines WM9 and WM164 was achieved by lentiviral transduction. Direct effects on protein levels of E- and N-cadherin were monitored by immunoblotting. To test for the functional effects of Slug and Twist, we performed adhesion, wound healing, migration, proliferation and soft agar assays. Upon Twist silencing, N-cadherin was significantly down regulated. This led to a reduced adhesion of melanoma cells to fibroblasts as well as to reduced migration.Slug silencing was followed by enhanced E-cadherin protein levels which subsequently resulted in a significant increase in adhesion to keratinocytes and to decreased migration. These results were confirmed by Slug over expression in two metastatic cell lines. In WM164, Slug over expression was followed by reduced E-cadherin levels and adhesion to keratinocytes. Further, migration was increased inWM9 and WM164 upon Slug over expression. These data demonstrate that cadherins are differentially regulated by Slug and Twist in melanoma. Slug affects adhesion to E-cadherin expressing keratinocytes, whereas Twist reveals a higher influence on adhesion to N-cadherin expressing fibroblasts. Additionally, both proteins play an important role in cell migration. The cadherin-specific effects of Slug and Twist sugget a sequence of EMTR- activation in melanomagenesis.

P247

Identification of SerpinB1 as a novel predictive molecular marker for chemosensitivity in melanoma

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For melanoma patients with distant metastases all available therapeutic options demonstrate only very limited efficacy up to date. This fact substantiates the need of predictive markers for therapy response. For example, ex-vivo chemosensitivity testing by an ATP-based luminescence assay is a promising tool to predict the individual outcome of different chemotherapy regimens. Indeed, this assay demonstrates a heterogeneous chemosensitivity against different cytotoxic drugs which correlates with chemotherapy outcome in terms of therapy response and overall survival; for the treatment of the patient the drug with the best individual chemosensitivity index (BICSI) is used. To circumvent this elaborate assay in the future, we want to identify and characterize predictive molecular biomarkers of specific chemosensitivity. Initially, predictive biomarker aspirants were identified by a microarray comparing chemosensitive and chemoresistant melanoma cell lines. Subsequently, we performed real time PCR on additional cell lines to confirm highly differentiated gene expression. To this end, we found Secernin 1 (SCRN1), Lysyl oxidase-like 1 (LoxL1), Thymosin beta 4 X-linked (TMSB4X), Vesicle-associated membrane protein 5 (Vamp5) and Serine protease inhibitor B1 (SerpinB1) as differential expressed in chemosensitive versus chemoresistant melanoma cells. Furthermore, we correlated the relative expression of our candidates with the chemosensitivity index of different chemotherapy regimes in 134 melanoma tissues so far. Importantly, we found a significant correlation between SerpinB1 expression and chemosensitivity towards Paclitaxel and Platin. Moreover, we also detected a differential expression of SerpinB1 in melanoma cell lines and tissue by immunohistochemistry. To sum up, SerpinB1 seems to be a promising biomarker for prediction of Paclitaxeland Platin chemosensitivity. Currently, we analyze the correlation of SerpinB1 protein expression with the *ex-vivo* chemosensitivity. In additional experiments, SerpinB1'srole for chemosensitivity will be analyzed in in-vitro experiments.

P248

The cytoskeleton seems to be involved in the E-cadherin dependent regulation of c-Jun in malignant melanoma

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The transcription factor c-Jun is a key player in the process of cell proliferation and tumor progression. It forms homodimers or heterodimers with other members of the transcription factor superfamily AP-1, influencing the expression of a multitude of regulators involved in tumor development and metastasis. In previous studies we could show that c-Jun protein is regulated via the cell-cell-adhesion molecule E-cadherin in malignant melanoma. Loss of E-cadherin leads to an induction of c-Jun protein expression. It is known that the expression of E-cadherin is important for the cytoskeletal organisation of melanoma cells, so we speculate if the cytoskeleton is involved in the E-cadherin dependent regulation of c-Jun. Immunofluorescence experiments with cytoskeletal disrupting agents give a clear hint that the cytoskeleton is involved in the regulation of c-Jun. RhoC, a member of RhoGTPase family, is important for cytoskeletal reorganisation. It is significantly overexpressed in melanoma and contributes to an enhanced metastatic phenotype of melanoma cells. Our experiments clearly show that RhoC participates in theE-cadherin dependent regulation of c-Jun, as inhibition of RhoC with a dominant negative construct leads to a significant decrease of AP-1 activity in melanoma cell lines. A main effector of RhoC seems to be the transcription factor Ets-1. It is well established that overexpression of Ets-1 leads to invasion of melanoma cells. Our analysis revealed that inhibition of Ets-1 results in a significantly down-regulation of c-Jun on translational level and of AP-1 activity via E-cadherin. We suggest that loss of E-cadherin in melanoma leads to reorganisation of the cytoskeleton and induction of the transcription factor Ets-1, which mediates overexpression of RhoC resulting in induction of c-Jun protein expression and AP-1activity. Unravelling the signaling cascade resulting in strong AP-1 activity in melanoma is important and will potentially lead to the identification of new targets for therapeutic intervention in melanoma.

P249

Induction of rapidly growing primary cutaneous melanomas in adultHgf-Cdk4R24C mice following a single epicutaneous carcinogen application

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We previously demonstrated that Hgf-Cdk4R24C C57BL/6 mice rapidly develop multiple primary cutaneous melanomas following neonatal exposure to the carcinogen dimethylbenzanthrene (DMBA) in a dose-dependent manner. To investigate whether melanomas could also be induced in adult Hgf-Cdk4R24CC57BL/6 mice we treated cohorts of 8–10 week old animals with DMBA according to a full carcinogenesis protocol. 400 n mol DMBA were applied epicutaneously on the shaved back on day 0 followed by 100 n mol DMBA on days 7, 14, 21 and 28. This regimen led to synchronous appearance of multiple, rapidly growing nodular melanomas between day 56 and 63. Mice had to be sacrificed between day 84 and98 after the initial carcinogen exposure due to large tumor burden. All animals showed metastatic spread of melanoma cells to the draining lymph nodes and lungs. The time course of tumor development, the histomorphologic appearance and the expression pattern of relevant melanoma genes resembled that observed in mice following neonatal carcinogen administration. Importantly, melanomas induced by carcinogen in adult mice also efficiently evaded recognition and destruction by innate and adaptive immune effector cells. Neonatal tolerance induction can not explain the immunological non-responsiveness observed in these primary tumors which should carry many potentially immunogenic mutations. We are currently also investigating the effect of a standard two step (DMBA/TPA) carcinogenesis protocol on melanoma development in Hgf-Cdk4R24C C57BL/6 mice and the results of these experiments will be reported.

P250

Endothelial cell-specific functions of RAGE signaling in inflammation and cancer

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Mechanisms of innate and adaptive immunity play a pivotal role in the development of cancer. Chronic inflammation can drive tumor development, but antitumor immunity can also restrict or even prevent tumor growth. Previously we have shown that feed-forward signals downstream of the receptor for advanced glycation end-products (RAGE) can fuel chronic inflammation, creating a microenvironment that is ideal for tumor formation. Mice deficient for the receptor for advanced glycation end-products (RAGE) are resistant to DMBA/TPA-induced skin carcinogenesis and show severely reduced inflammatory response to treatment with TPA accompanied by impaired infiltration with subsets of innate immune cells and impaired up-regulation of pro-inflammatory genes. Interestingly, tumors in RAGE-deficient mice showed severly reduced vascular density and impaired perfusion using confocal microscopy and perfusion-ultrasound-sonography. Furthermore, aortic rings explanted from RAGE-deficient mice were characterized by impaired micro vessel out growth revealing an important role of RAGE signaling in endothelial cell migration and endothelial progenitor cell recruitment. In order to elucidate an endothelial cell-specific role of RAGE signaling, we generated wild type bone marrow chimericTie2 promoter-driven RAGE-deficient mice (RageΔend) that show a significantly altered inflammatory response to TPA treatment characterized by impaired immune cell recruitment in the presence of epidermal hyperplasia thereby partly phenocopying non-conditional RAGE-deficient mice. In conclusion, we demonstrate the complex role of RAGE signaling in driving the strength and maintenance of an inflammatory reaction during tumor-promotion and in promoting tumor angiogenesis. Moreover, we provide direct genetic evidence for a novel endothelial cell-specific function of RAGE in promoting inflammation-induced skin cancer.

P251 (V35)

Cyclosporine A inhibits DNA repair by a calcineurin-mediated mechanism

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The calcineurin-inhibitor cyclosporin A rather than other immunosuppressive drugs like the mTOR-inhibitor everolimus leads to an increased skin cancer risk, e.g. in organ transplant patients. We have recently shown that cyclosporin A but not everolimus inhibits DNA repair which can lead to increased UV-induced carcinogenesis. To investigate, whether this effect is mediated by the calcium/calmodulin-dependent phosphatase calcineurin or via calcineurin-independent mechanisms we assessed the influence of calcineurin on cellular DNA repair using a functional test system, the host cell reactivation (HCR)assay in GM00637 fibroblasts. Transfection of an expression plasmid for the catalytic calcineurin subunit calcineurin A led to a significant dose-dependent enhancement of the cellular DNA repair capacity up to 230% ($P < 0.0005$). In contrast, incubation of the cells with 0.1 μM cyclosporin A resulted in a significant inhibition of the cellular DNA repair capacity to 82% ($P < 0.005$). Simultaneous over expression of calcineurin A completely compensated the inhibitory effect of cyclosporin A and led to normal DNA repair in cyclosporin A-treated cells. Moreover, the transfection of two different calcineurin A RNAi constructs resulted in significant inhibition of cellular DNA repair to 78% ($P < 0.0005$), which was comparable to the effect of cyclosporin A. The inhibitory impact of the two RNAi on calcineurin A protein expression was confirmed by western blot analysis. As calcineurin is a calcium-dependent phosphatase we additionally assessed the influence of calcium on DNA repair. Incubation of the cells with the calcium-ionophor A23187 resulted in significant increase of cellular DNA repair up to 128% ($P < 0.05$). These results demonstrate that calcium and calcineurin modulate cellular DNA repair and that the inhibitory effect of cyclosporin A on DNA repair is mediated by calcineurin. We propose that calcineurin is an important regulator in UV-induced carcinogenesis under a cyclosporin A-therapy.

P252

Hyaluronan is deposited in tumor stroma under control of the tumor-involvement of the MAPK-signaling pathway

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Tumor-stroma-Interactions are thought to be important for tumor growth and metastasis. Besides many other factors the hyaluronan (HA) metabolism of the fibroblasts (FB) is affected. Our previous data suggest that FB are stimulated by cells of the Malignant Melanoma (MM) to deposit large amounts of HA in the tumor stroma, thus supporting MM cell proliferation and motility. In the present paper we analyzed (i) the expression pattern of cultured FB treated with MM cell conditioned medium concerning HA-Synthetases 1 and 2 by RT-qPCR (ii) as well as western blot.(iii)The resulting amount of deposited HA was measured by HA-ELISA. (iv)Blocking experiments were performed to discover the factors responsible for the changed HA deposition of FB.

Results: Medium transfer experiments showed that MM cells secrete soluble mediators that induce HAS1- and HAS2 expression in FB resulting in a significantly higher HA-secretion by FB. We could show that TGF-β1 is an inductor of HAS1mRNA and protein expression. The factor inducing HAS2 in FB is not yet identified. Experiments using proteolytic treatments showed that the inducing agent is a protein and not a metabolic agent of the MM cells, like lactate. Inhibitor experiments targeting various elements of the MAPK-signaling pathway proofed that this signal cascade is essential for HAS2-induction by MM-derived mediators in the fibroblasts. Here we present putative inducers like PDGF-BB, PDGF-C and IL-1β who could play the key role in the increased HA expression of the MM and its surrounding stroma.

These experiments outlined herein not only establish a model to study tumor promoting tumor-stroma-interactions but may also identify novel targets for anti-proliferative or anti-metastatic therapies in malignant melanoma.

P253

The lymphatic marker podoplanin influences the cell cycle in squamous cell carcinoma

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Podoplanin is a small transmembrane glycoprotein binding lymphatic endothelial cells specifically. It promotes cell development and migration by interacting with ezrin and moesin. These proteins link membrane proteins to the actin cytoskeleton. The expression of podoplanin is induced by EGF, FGF2 and TNF-α and is upregulated in many different human cancers, e.g. squamous cell carcinomas. Recent studies have shown that the transcription factor c-fos binds to the promoter region of podoplanin and activates its transcription. Further studies have shown that podoplanin is able to induce invasion of single cells after loss of E-cadherin and to induce collective cell migration. Podoplanin is expressed in the invasive front of the migrating cells. However, the molecular mechanisms podoplanin is involved in are still unknown. We investigated podoplanin using microarray studies of lymphatic endothelial cells treated with podoplanin siRNA. One significantly downregulated gene in siRNA treated cells was cyclin E. The protein of this gene has been described to participate in oral carcinogenesis determining the restriction point of the G1 phase of the cell cycle. Downregulation of podoplanin reduces the expression of cyclin E inducing a cell cycle arrest of cells in the G1 phase. Correspondingly, we found human carcinoma cells Cal 39 treated with podoplanin siRNA arrested in the G1 phase of their cell cycle. Moreover, reduction of podoplanin in Cal39 cells reduced their capacity for proliferation shown by BrdU and ATP-Luciferase assays. These results lead to the assumption that the upregulation of podoplanin induces cyclin E playing a critical role in progression of squamous cell carcinoma.

P254 (V03)

Merkel cell polyoma virus T antigen induces survivin and is required for the tumor phenoptype of infected Merkel cell carcinoma cells

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Human Merkel cell polyoma virus (MCV), a common but previously unrecognized human infection, is clonally-integrated into ~80% of Merkel cell carcinomas (MCC), analogous to papilloma virus integration in cervical carcinomas. Direct evidence for whether MCV causes MCC can be obtained from viral gene knock down studies using MCV-positive MCC cell lines. To address this, we knocked down MCV T antigen expression in MCC cell lines using three different shRNA-expressing vectors targeting exon 1 of the T antigens. MCC cell lines used include four newly generated cell lines from MCC tumors, three of which (WaGa, BroLi, MS-1) carry stably integrated MCV DNA. All MCV-positive MCC cell lines underwent growth arrest and/or widespread cell death upon T antigen knock down, whereas proliferation of MCV-negative cell lines remained unaffected. Although modest increases in Annexin-V+/7-AAD-cells were observed upon T antigen knock down in some MCV-positive cells, activation of caspases or changes in expression and phosphorylation of Bcl-2 family members were not consistently detected after T antigen suppression. Knock down of T antigen, however, was directly correlated with loss of survivin protein expression, which is expressed more abundantly in MCV-positive compared to MCV-negative cell lines. These results demonstrate that MCV T antigen expression is required for survival of MCV-positive cell lines. Loss of MCV T antigen in MCV-positive cells results in growth arrest and/or cell death that is correlated with loss of survivin on coprotein expression.

P255

Beta-catenin overexpression in primary melanocytes induces adhesion-independent cell death

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Beta-catenin has multiple tasks in a cell. Together with transcription factors of the TCF/LEF family it regulates the transcription of various genes, such as C-JUN, MITF and cadherins. At the cell membrane, it serves as a linker between cadherins and the cytoskeleton. It is also known that beta-catenin plays a role during chromosome segregation and centrosome separation. Beta-catenin plays a major role in different types of cancer and is overexpressed during melanoma progression. We could show that beta-catenin is an essential survival factor for metastatic melanoma cells. In contrast, primary melanocytes and radial growth phase melanoma cells do not need beta-catenin for survival or proliferation. To analyze the role of beta-catenin in melanoma development, we overexpressed constitutive active beta-catenin in primary melanocytes or radial growth phase melanoma cells by adenoviral gene transfer. Overexpression of beta-catenin resulted in activation of TCF/LEF/beta-catenin mediated transcription of target genes and loss of E-cadherin expression in primary melanocytes. Furthermore beta-catenin overexpressing melanocytes and radial growth phase melanoma cells lost their adhesion to the culture plate and stopped to proliferate. Interestingly, the cells survived in this detached state for 3 days and died subsequently. Microarray analysis of primary melanocytes expressing the constitutive active beta-catenin revealed changes in expression of genes involved in cell adhesion, differentiation and cell survival, as well as chromosome segregation. Immunohistochemical analysis revealed that primary melanocytes and radial growth phase melanoma cells overexpressing beta-catenin are not able to form a proper mitotic spindle which prevents a separation of the chromosomes during mitosis und thus a division of the cell. We suggest that the observed cell death occurs due to a mitotic catastrophe triggered by the failure of the mitotic spindle to form. Therefore, expression of beta-catenin in benign melanocytic cells and melanoma cells is tightly regulated to ensure proper survival.

P256

Deciphering the role of the Y-box binding factor 1 in malignant melanoma

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Y-box binding protein 1 (YB-1) is a member of the cold shock domain protein family and regulates transcription and translation by binding to RNA and DNA. YB-1controls the expression of genes involved in cell proliferation, migration, DNA replication and drug resistance. Our own data show that YB-1 expression is upregulated and the protein is translocated to the nucleus during melanoma progression, and that YB-1 is an important transcription factor regulating proliferation, survival, migration, invasion and chemosensitivity of melanoma cells. However, the mechanisms governing the expression and activity of YB-1 during melanoma progression are still not known. To identify the factors which regulate the expression, nuclear translocation and functional activities of YB-1 in melanoma cells we tested the effect of several signal transduction inhibitors, stress stimuli and growth factors on YB-1 promotor activation, YB-1 expression and nuclear translocation and phosphorylation. Our data indicate that in particular microenvironmental survival factors and the PI3K/AKT signalling pathway play a critical role in YB-1 activation in melanoma cells. Furthermore, to elucidate the molecular mechanisms by which YB-1 affects melanoma progression, we downregulated YB-1 expression by lentiviral delivery of shRNA in several metastatic melanoma cell lines. Interestingly, YB-1 downregulation in several melanoma cell lines resulted in reduced survival and proliferation, however the effect was seen only more than 1 week after lentiviral transduction. These data suggest that melanoma cells can in the beginning compensate for the loss of YB-1 expression, but later on melanoma cell survival and proliferation seems to be dependent on YB-1 expression and function. Further studies will indicate to what extent YB-1 is involved in melanoma progression and whether YB-1 may be a molecular target in melanoma therapy.

P257 (V02)

Beta-catenin is an essential survival factor in metastatic melanoma cells

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The regulation of beta-catenin expression and nuclear translocation plays a key role in embryogenesis and cell proliferation. Stabilization or nuclear translocation of beta-catenin has been observed in many types of cancers, such as colon, lung, skin, breast, liver and pancreas cancers. Nuclear translocation of beta-catenin is followed by binding to the T cell factor/lymphocyte enhancer binding factor family (TCF/LEF) and activation of the expression of genes involved in cell proliferation and survival. In malignant melanoma there are contradictory results concerning the role of beta-catenin in tumor progression. Whereas several studies propose that an increased nuclear translocation and activity of beta-catenin in melanoma cells promote tumor proliferation, others found recently that elevated levels of nuclear beta-catenin correlate with improved survival from melanoma and that beta-catenin downregulation promotes metastases formation in mice.

Our data indicate that in the course of melanoma progression from the radial to the vertical growth phase to metastatic melanomas the expression and nuclear translocation of beta-catenin increases. We investigated the biological effects of inhibiting beta-catenin using either small molecular antagonists of active beta-catenin, inducible shRNAs against beta-catenin or by activating the degradation of beta-catenin. Interestingly, blockade of beta-catenin induces efficiently apoptosis, inhibits proliferation and migration and decreases chemoresistance *in vitro* in metastatic melanoma cells. In addition, subcutaneous melanoma growth in SCID mice was almost completely inhibited by an inducible beta-catenin downregulation. Interestingly, inactivation of beta-catenin degradation in radial phase melanoma cells induces a more aggressive phenotype, enabling the cells to invade and migrate. These data indicate that beta-catenin is an essential survival factor in metastatic melanoma cells.

P258

Persistent NFkappaB activation in malignant melanoma is generated through loss of the tumor suppressor CYLD

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NFkappaB activation has been connected with multiple aspects of melanoma development but the underlying molecular mechanisms of persistent NFkappaB activation in tumors remain largely unknown, so far. Recent studies suggested a role of CYLD (identified as a tumor suppressor that is mutated in familial cylindromatosis) in deubiquitinating the NFkappaB coactivator Bcl-3, thereby preventing its translocation into the nucleus, where Bcl-3 normally interacts with NFkappaB and activates transcription.

Thus, we evaluated CYLD transcription in different melanoma cell lines and performed immunohistochemical analysis of a tissue microarray consisting of 88 primary human melanomas.

CYLD was downregulated or lost in all tumor cell lines investigated as compared to primary human melanocytes. The *in vivo* analysis revealed reduced CYLD expression in the tumor samples of patients compared to non-tumorous tissue and in particular we found a significant correlation regarding tumor invasiveness and tumor thickness after loss of CYLD expression.

We further analysed the signalling pathway regulated through loss of CYLD. As a direct consequence of CYLD repression, Bcl-3 translocates into the nucleus and activates transcription together with the NFkappaB subunits p50/p52. Targets of the transcriptional activity are the cyclinD1 and N-cadherin promoters, resulting in proliferation and invasion of melanoma cells. Rescue of CYLD expression in melanoma cells reduced proliferation and invasion *in vitro* and tumor growth and metastasis *in vivo*.

Until today, it becomes generally accepted that melanocytes transformed into malignant melanomas through the interplay of genetic factors/genetic mutations and the ultraviolet (UV) spectrum of sunlight. Therefore, our recent findings center on UVB dependent regulation of CYLD. Our data showed a UVB dependent transcriptional downregulation of CYLD in human melanocytes. Regarding this, we analyse the signalling pathway triggered by sun irradiation and resulting in CYLD repression in the human skin.

P259

Identification of a gene expression signature of sentinel lymph nodes in cutaneous melanoma

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Cutaneous melanoma first metastasizes into sentinel lymph nodes that control the lymphatic drain from the area of the primary tumor. Sentinel lymph node biopsy has therefore become an important diagnostic procedure in patients with primary melanomas at tumor stages ≥T2a. Successful colonization of tumor cells into sentinel lymph nodes is thought to require priming of this environment, but these priming factors are yet undefined.

Using our previously described xeno transplantation model, in which human melanoma cells injected into the skin of SCID mice metastasize to sentinel lymph nodes, we aimed to identify and analyze a gene expression signature reflecting priming events. Tumor negative sentinel lymph node expression profiles have been compared to expression profiles i) of micro- and macro-metastases and ii) of lymph nodes of control (= non tumor-bearing) animals. For analysis, Affymetrix® gene arrays have been used. Results obtained from these experiments have been validated by using RT-PCR based mRNA analysis and immunohistochemistry. With these techniques we were able to identify gene products which may serve as potential candidates responsible for initiating melanoma lymph node metastasis by altering the lymph node microenvironment.

P260 (V11)

The neuronal transcription factor Brn3a: essential for human melanoma cell proliferation and survival

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Melanocytes and neuronal cells both originate from the same precursor cells located in the neural crest. Melanoma derives from the malignant transformation of melanocytes and neuronal proteins can be expressed in melanoma. Here we describe the POU domain transcription factor Brn3a, normally involved in development of sensory neurons, to be highly expressed in melanoma cell lines (~90%) and in primary tumors. In contrast, Brn3a was not expressed in melanocytes and melanocytic nevi. Targeting Brn3a by RNA interference strongly reduced viability of melanoma cell lines and decreased tumor growth *in vivo*. Silencing of Brn3a induced cell cycle arrest in the G1 phase followed by apoptosis. Critical for cell cycle arrest was the tumor suppressor p53 that was activated via active DNA damage signaling. Loss of Brn3a in melanoma caused DNA double-strand breaks as detected by Mre11/RAD50-containing nuclear foci and led to DNA damage signaling followed by p53 activation. A genome-wide expression screen revealed the mitotic spindle motor protein Kif11 as one target gene of Brn3a in melanoma. Similar to Brn3ainhibition, silencing of Kif11 in melanoma cells resulted in DNA double-strand breaks and activated DNA damage signaling and p53-mediated G1 phase arrest in a subpopulation of cells. These results identify Brn3a as a new survival factor in melanoma, required for tumor cell proliferation by regulating molecules essential for cell cycle progression. Thus, Brn3a has high potential as a new biomarker and therapeutic target for melanoma.

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Patient survival, tumor stage and depth of malignant melanoma are associated with distinct expression profiles: A high-throughput-tissue microarray-based study

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Cutaneous malignant melanoma represents the leading cause of skin cancer death in industrialized countries. Clinical and histological variables that predict survival, such as Breslow's index, tumor size, ulceration, or vascular invasion have been identified in malignant melanoma. However, the potential relevance of biological variables still needs to be analyzed. Using tissue microarrays (TMAs), we retrospectively analyzed samples from 465 patients; 364 with primary malignant melanomas, 39 with metastases, and 62 with benign nevi. Clinical follow-up data (AJCC 2002 staging, overall and progression-free survival and tumor therapy) provided by the Central Tumor Registry Regensburg, were available for all patients with primary malignant melanomas. The median follow-up for all patients with malignant melanoma was 51.5 months (range 6–186 months). A panel of 72 different antibodies for cell cycle, apoptosis, melanoma antigens, transcription factors, DNA mismatch repair, and other proteins was used. After data preprocessing, three different target variables were chosen for further analyses: First, a Bayesian Lasso method was used with a Binomial likelihood model for discrimination between two classes (pT1/2 versus pT3/4). Second, a regression using tumor depth as target variable was calculated based on a Gaussian likelihood model according to the standard linear model. Finally, a regression using overall survival as target variable was computed using a Weibull likelihood model for predicting the survival times. In particular, all three models identified loss of expression of P-Cadherin and of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) as best predictors for advanced stage, increased tumor depth and shorter overall survival. Further, consistent negative patterns occurred in all experiments for BCL2L1 and BAX overexpression, however, only after conditioning on a second variable (HMB45 for tumor stage; Ki67 for survival). The identification of such expression profiles by mathematical modeling may distinguish specific melanoma progression stages and improve predictive evaluations for patients with melanoma.

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Myeloid-derived suppressor cells: a critical immuno-regulatory mechanism - not only for tumor control

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Recent studies have described the so-called myeloid-derived suppressor cells (MDSC) in various tumors. They suppress anti-tumor CD8+ T cell responses and represent a newly detected important immune escape mechanism of tumors. In mice they can be phenotypically identified by cell surface expression of CD11b, Gr-1 andIL-4 receptor alpha (CD124).

We demonstrated the presence of MDSC in a murine tumor model of B16 melanoma.B16- induced MDSC express MHCII molecules on their surface, thus they are able to present antigens, but they down-regulate antigen-specific CD4+ T cell responses.

We now show that the presence of MDSC is essential to down-regulate not only anti-tumor immune responses, but also to negatively modify immune responses directed against infectious agents.

Therefore we examined melanoma-bearing mice that were additionally infected with Leishmania major (L. major) subcutaneously. Tumor-bearing mice showed a significantly weaker anti-L major CD4+ response than non tumor-bearing mice. As a critical control, we used S100A9 knock-out mice that are known to lack functional MDSC (Cheng et al., 2008). These mice, when tumor-bearing showed normal CD4+immune responses upon s.c. infection with L. major.

Our data show that the existence of MDSC might be a critical mechanism of immunosuppression in tumor patients and that therapies targeting MDSC might be beneficial to restore anti-tumor responses.

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Melanoma cells are capable of activating the coagulation machinery

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Upon haematogenous spreading, a plethora of tumor cells was reported to elicit a tumor-associated activation of blood coagulation. This procoagulant activity does not only bear the risk of thromboembolism but also facilitates tumor dissemination and formation of metastasis. A growing body of evidence indicates that therapies facing mechanisms that contribute to the hypercoagulability associated with cancer bear a beneficial impact for the patients' outcome. In our study we focused on the mechanisms by which melanoma cells trigger procoagulatory conditions and on the impact of heparins in this context. Melanoma cell lines representing different growth phases (RGP, VGP, MM) expressed on their surface tissue factor (TF), a key component of the coagulation pathway activating thrombin. Although the expression levels varied, both the low TF- and high TF-expressing metastatic cells (i.e. MV3 andWM9, respectively) were very potent to induce an immediate thrombin generation in blood plasma. TF, but not previously described cancer procoagulant (CP), turned out as the major player in this process. Thrombin targets protease activated receptor 1 (PAR-1), whose cleavage on endothelial cells (EC) leads to the instantaneous release of vasoactive von Wille brand factor (VWF). Additionally, some metastatic melanoma cells lines (MV3 or BLM) were able to induce VWF release directly by thrombin-independent pathway. As shown for MV3 cells, that thrombin-independent way of EC activation involves cleavage of PAR-1 by melanoma-secreted metalloproteinase 1 (MMP-1). Upon flow condition representing the blood stream, VWF builds a network ready to bind platelets, the prerequisite for thrombus formation. Moreover, due to its highly adhesive properties, luminally exposed ultra-large VWF (ULVWF) seems to represent a central link between circulating cells including melanoma cells and vascular EC. Low-molecular-weight heparins (LMWHs), next to their ability to inhibit thrombin generation mediated by melanoma cell-expressed TF, showed an additional protective effect on melanoma-induced direct EC activation as measured by diminished VWF release in response to MV3supernatant. Our data show that melanoma cells execute procoagulatory effects on EC via thrombin-dependent and -independent pathways. We postulate that LMWHs may have promising effects for melanoma patients due to preventing tumor-induced thrombotic conditions facilitating tumor cell spreading.

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Tryptase and histamine up regulate VEGF and PDGF in squamous cell carcinoma cells (SCC)

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The accumulation of mast cells (MC) in peri-tumorous tissue has been known for many years. Several immunohistochemical studies indicated that MC might regulate tumor progression via secreted cytokines, growth factors or mediators like histamine or tryptase. In our previous studies we were able to demonstrate that mast cell derived TNF alpha induced production and release of IL-6 and IL-8 in melanoma and SCC lines. These results further prompted us to check, whether mast cell derived mediators like histamine or tryptase may be able to regulate growth factor expression by tumor cells, thus potentially modulating tumorigenesis and progression.

Various squamous cell carcinoma cell lines were cultivated for 24 h with or without conditioned medium derived from IgE-activated or non-activated primary, dermal mast cells. Protein expression of growth factors bFGF, VEGF and PDGFA was estimated by ELISA in each individual cell line in order to look for a potential modulation of these growth factors as a result of tumor-mast cell interaction. Furthermore, tumor cells were stimulated with the mast cells related mediators histamine or tryptase to examine how these mediators would influence expression of bFGF, VEGF and PDGF in tumor cells.

In all examined cell lines, low levels of bFGF protein were detectable, but not altered by co-cultivation with supernatant derived from activated or non activated mast cells. In contrast to bFGF, PDGF was released in elevated amounts after co-cultivation of SCC cell lines with mast cell supernatant. Interestingly, the modulatory effect of supernatant derived from activated versus none activated mast cells on PDGF release from tumor cells differed only slightly. Stimulation of SCC cell lines with low concentrations of tryptase (0.1 μg/ml) led to a strong increase in PDGF release (in SCC-12 cells from 32 pg/ml to 150 pg/ml and in SCC-13 from 18 pg/ml to 92 pg/ml).This result suggests that tryptase may be responsible for the effect of mast cells on PDGF release from SCC cells. Similar results were obtained for VEGF release from SCC lines after cultivation with mast cells supernatant, histamine or tryptase (in SCC-12 cells from 218 pg/ml to 283 pg/ml and in SCC-13 from 86 pg/ml to 285 pg/ml).

Our results indicate that mast cell-descendent mediators like tryptase and histamine are strong modulators of VEGF and PDGF in tumors cells and may play a supportive role for the progression of cutaneous tumors.

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Dendritic cells and macrophages upregulate the chemokine CCL18 in mycosis fungoides

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Mycosis fungoides (MF) is associated with strong cutaneous dendritic cell (DC) and macrophage infiltration that to some extent supports tumor cell survival but can also stimulate the immune defense reaction against tumor infiltration. The interaction of DC's with tumor cells is partially mediated by chemokines which play important roles in tumor pathogenesis and metastasis. PCR analysis of chemokine expression in lesional MF skin from patients in patch and tumor stage revealed, that similar to the previously described upregulation of CCL17, mRNA expression of the chemokine CCL18 is increased in MF skin compared to healthy tissue. In line with these data, CCL18 protein expression was increased up to 3 times in sera of MF patients compared to healthy controls. To further investigate the role of CCL18 in lymphoma pathogenesis we analysed expression of chemokines *in situ* in relation to distribution of the different dermal DC types by three color immunofluorescence staining. In patch stage of MF CCL18 was expressed by dermal CD163+ CD14+ macrophages and CD1c+ CD1a+ CD11c+ DC's of the dermis and epidermis. In tumor lesions CCL18 expression was mainly detected in CD209+ macrophages located at the tumor invasion front. Mature CD208+ CD1c+ DC's located in the center of tumor cell aggregations did not express CCL18 but costained with CCL17. In contrast to CCL18, CCL17 was expressed by fibroblasts and antigen presenting cells located in close contact with CCR4+ tumor cells in patch and tumor stage MF lesions. This pattern suggested that CCL18 secreted by DC's and macrophages functions as a local antitumor immunomodulator whereas CCL17 expression might rather mediate accumulation of CCR4 expressing lymphoma cells in the skin.

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New caspase-independent but ROS-dependent apoptosis pathways are targeted in melanoma cells by an iron-containing cytosine analogue

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Chemotherapy resistance and related defects in apoptotic signaling are crucial for the high mortality of melanoma. Effective drugs are lacking, also due to the fact that apoptosis regulation in this tumor is essentially not understood. The cytosine analogue ferropoptoside (N69), which contains an iron carbonyl complex, resulted in strong induction of apoptosis in melanoma cells starting already after 2 h, where as cytotoxicity remained at a low level. Surprisingly, there was no indication for any caspase activation at early times, although cytochrome c was released from mitochondria. Indicative for new proapoptotic pathways was the production of reactive oxygen species (ROS) as an early effect, and the inhibition of apoptosis by the antioxidant vitamin E. Apoptosis was also blocked by exogenous Bcl-2 overexpression and by the pan-protease inhibitor zVAD. However, only zVAD also prevented ROS production, for which Bcl-2 remained without an effect. Thus, new proapoptotic pathways are described here for melanoma cells clearly related to ROS production. A cascade enclosing enhanced levels of intracellular iron, which lead to enhanced ROS production in a Fenton reaction, appears as suggestive. Where as off-target effects of zVAD appear as upstream, Bcl-2 may exert its inhibitory activity downstream of ROS. New proapoptotic pathways are of particular interest formelanoma as they may open new options for targeting this highly therapy-refractory tumor.

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Oncogene-induced senescence of primary human melanocyte

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Oncogene-induced senescence acts as a barrier against tumor formation and has been implicated as the mechanism preventing the transformation of benign melanocytic lesions that frequently harbour oncogenic B-RAF or N-RAS mutations. In the present study we systematically assessed the relative importance of the tumor suppressor proteins p53, p21Waf1, pRb and p16INK4a in mediating oncogene-induced senescence in human melanocytes. We now show that oncogenic N-RAS induced senescence in melanocytes is associated with DNA damage, a potent DNA damage response and the activation of both the p16INK4a/pRb andp53/p21Waf1 tumor suppressor pathways. Surprisingly neither the pharmacological inhibition of the DNA damage response pathway nor silencing of p53 expression had any detectable impact on oncogene-induced senescence in human melanocytes. Our data indicate that the pRb pathway is the dominant effector of senescence in these cells, as its specific inactivation delays the onset of senescence and weakens oncogene-induced proliferative arrest.

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The R248C FGFR3 hotspot mutation affects cell growth, apoptosis and attachment of human HaCaT keratinocytes

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FGFR3 mutations have recently been identified in several benign epidermal skin tumors such as seborrheic keratosis, epidermal nevus and solar lentigo. The functional consequences of these mutations in human skin are yet unknown. In this study we analyzed the functional effects of the most common FGFR3 mutation in skin, the R248C FGFR3 hotspot mutation, in human HaCaT keratinocytes. The cells were stably transduced with either the R248C or wildtype FGFR3 IIIb sequence using a retroviral vector system. FGFR3 mutant and wildtype cells showed similar growth rates at a cell density of 50%. However, at confluence FGFR3 mutant keratinocytes revealed a significant higher cell growth than wildtype cells ($P < 0.01$). Furthermore, FGFR3 mutant cells showed a significantly decreased apoptotic rate assessed by caspase 3/7 assay ($P < 0.001$) and a significantly decreased attachment to fibronectin ($P < 0.001$) compared with FGFR3 wildtype cells. The mutant HaCaT keratinocytes did not show a significantly different migration capacity and beta-galactosidase activity as a marker for senescence. Gene expression array revealed only a few differentially expressed genes between FGFR3 mutant and wildtype keratinocytes. The analysis of signaling pathways demonstrated an enhanced phosphorylation of ERK1/2 and PLC-gamma in confluent R248C mutant HaCaT cells compared with wild type keratinocytes. In this study we demonstrate for the first time that the R248C FGFR3 hotspot mutation affects cell growth, apoptosis and attachment in human keratinocytes. Our results suggest that an increased cell growth at density along with a decreased apoptosis rate may contribute to the development of acanthotic tumors in FGFR3 mutant skin *in vivo*.

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Can therapies targeting the tumor vasculature inhibit tumor growth?

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As tumor growth strictly requires the formation of new blood vessels, various anti-angiogenic strategies are therapeutical approaches to prevent solid tumor growth. Here, we targeted the vascular endothelial growth factor (VEGF)/VEGF-receptor system that is considered to be essential for tumor angiogenesis, focussing on endothelial receptor tyrosine kinase VEGFR-2. In most adult vascular beds VEGFR-2 is downregulated while it is strongly expressed in growing blood vessels, namely the tumor endothelium. Therefore, gene regulatory elements of the VEGFR-2 gene are ideally suited to deliver toxins specifically to the tumor vasculature, minimizing the risk of systemic side effects.

Here, we generated transgenic mice, expressing the potent chimeric suicide gene yeast super-cytosine deaminase (super-yCD) under the control of distinct promoter/enhancer sequences of the murine VEGFR-2 (Flk1) gene, specifically active in the tumor endothelium. Conversion of non-toxic 5′-Fluorocytosine (5′-FC) to cytotoxic 5′-Fluorouracil (5′-FU) by super-yCD causes death of super-yCD expressing endothelial cells. Due to the robust bystander effect, 5′-FU also kills neighbouring tumor cells. Surprisingly, Flk1/super-yCD transgenic mice showed normal tumor growth after 5′-FC treatment. When we analyzed this surprising finding, the number of dilated tumor vessels was reduced by 50% in 5′-FC treated mice. Electron microscopy of untreated tumors revealed blood vessel morphology with highly activated endothelium typical for tumor tissue, including single necrotic and apoptotic endothelial cells (EC). In sharp contrast, the vascular bed of 5′-FC treated tumors had a phenotype closely resembling normal tissue vasculature with quiescent EC. Inline with the observed morphological changes, expression of Angiopoietin-2 was10fold decreased in 5'-FC treated tumors. Further studies will analyze the effects of this endothelium-specific tumor therapy on invasion and metastasis of experimental tumors. As first results in the literature suggest that anti-angiogenic therapies may increase the aggressivity of tumor cells, the results of our studies might have important impact on the development of novel anti-angiogenic therapies.

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Tumor-specific T helper 1 cells prevent malignant transformation

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Most cancer immuno-therapies rely on CD8+ killer T cells (CTL), capable of causing either tumor cell lysis or apoptosis. Despite potent killing of tumor cells under *in vitro* conditions, CTL often fail to efficiently eradicate tumors *in vivo*. Surprisingly, a small number of studies reproducibly showed that adoptive transfer of interferon gamma producing CD4+ T cells (Th1 cells) efficiently clears tumor diseases that are resistant to CTL-mediated rejection. Even though incapable of directly recognizing MHC-class II negative cancer cells, Th1 cells are essential for arresting tumor growth and aberrant angiogenesis, in the absence of detectable tumor cell killing.

To elucidate the mechanisms underlying the tumor growth arrest by Th1 cells, we investigated the development of islet carcinomas, resulting from aberrant Rb and p53expression. Following the expression of differentiation antigens (DA) over time in this model of endogenous multistage carcinogenesis, we first found that tumor-specificTh1 cells reduce cell proliferation as determined by BrdU labeling *in vivo*, without causing cell death or apoptosis. We next followed the expression of tissue specific DA acquired during embryogenesis. Interestingly, tumor cells reproducingly showed an ordered loss of DA, first glucose transporter 2 (Glut2) that is expressed at the final embryogenic phase A and consecutively insulin, acquired during midterm embryogenesis. In sharp contrast, DA expressed early during embryogenesis, such as synaptophysin remained strongly expressed, even on fully degenerated cancer cells. All three DA remained normally expressed and fully functional in islet cells of tumor prone mice treated with Th1 cells. Thus, besides killing, prevention of malignant transformation may be a major mechanism, responsible for therapeutic tumor immune surveillance.

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Evaluation of vascular patterns in various skin neoplasms using *in vivo* reflectance confocal microscopy

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Reflectance confocal microscopy (RCM) represents a novel non-invasive imaging technique that allows the *in vivo* examination of the skin in real-time. RCM has been used for the diagnosis of a variety of skin neoplasms including actinic keratosis, basal cell carcinoma and malignant melanoma with high sensitivity and specificity. However, the evaluation of different vascular patterns of cutaneous tumors has not yet been performed. Here, we describe the RCM features of vessels in Bowen's disease (BD), actinic keratosis (AK), basal cell carcinoma (BCC), lymphomatoid papulosis and malignant melanoma. A total of 10 patients have been included in this study. RCM results were correlated to conventional H&E histology. In AKs bright blood vessel dilation and elongation was observed. RCM evaluation of BD revealed a distinct vascular pattern with increased blood vessels that showed marked elongation, tortuosity and dilation of vessels and was observed in a regular pattern throughout the entire lesion. In BCCs large dilated blood vessels were prominent in association with tumor islands. RCM evaluation of lymphomatoid papulosis showed vessels with a prominent vessel wall and the presence of bright cells within these thickened walls. In malignant melanoma increased number of vessels as well as dilation and tortuosity was observed.

Overall, good correlation between RCM and histology was obtained. RCM allows the *in vivo* examination of tumor vessels and therefore the evaluation of dynamic patterns. In contrast to H&E tortuosity of the vessels, blood flow and lymphocyte rolling can be observed.

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cFLIP isoforms inhibit death receptor-induced NF-κB activation in primary and transformed keratinocytes

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Death receptors such as CD95 and TRAIL-R1/R2 induce apoptosis in many cells, but can also activate non-apoptotic signalling pathways (NF-κB as well as mitogen-activated protein kinases (JNK, p38). Different isoforms of FLIP (cFLIPS and cFLIPL) inhibit different steps in death receptor (DR)-associated activation and maturation of procaspase-8. We reasoned that the cleavage of cFLIP, in turn, could differentially influence nonapoptotic DR signals. Thus, we established stable HaCa Tcells expressing different cFLIP isoforms (cFLIPS, cFLIPL) or mutants of cFLIPL that are either uncleavable by capase-8 (cFLIPD376N) or generated after stimulation by DISC-associated caspase-8-mediated cleavage (cFLIPp43). All isoforms/mutants of cFLIPL blocked death ligand (DL)-mediated apoptosis, whereas a distinct cleavage pattern of caspase-8 was detected in the DISC. Only cells expressing full length cFLIPL (irrespective of cFLIP cleavage) sufficiently induced proteolysis of caspase-8 to its p43/41 fragments. In contrast, cFLIPS or cFLIPp43 blocked procaspase-8 cleavage.

When we examined DR-induced non-apoptotic signals, TRAIL or CD95L activated JNK within 15 minutes. MAPK p38 was induced in a biphasic manner. Interestingly, all cFLIP isoforms/mutants completely inhibited the late DL-induced activation of p38or JNK. Moreover, cFLIP isoforms or mutants blocked DL-mediated IκBα phosphorylation, NF-κB activation, and induction of the target gene IL-8.

Conversely stable knockdown of cFLIP isoforms in primary human keratinocytes not only resulted in increased apoptotic cell death but also enhanced DL-induced NF-κB activation and concomitant IL-8 induction, further supporting the physiological relevance of cFLIP isoforms for these DL-induced signals.

In summary, cFLIP isoforms are not only potent inhibitors of DL-induced apoptosis, but also block DL-triggered activation of NF-κB. The inhibition of non-apoptotic signalling by CD95 and the TRAIL death receptors by FLIP proteins might be of crucial importance during tumorigenesis of keratinocyte skin cancer in order to avoid activation of innate or adaptive immune responses in tumor cells acquiring apoptosis resistance.

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Spotlight on KIT mutations in mucosal melanoma

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Introduction: KIT is a receptor tyrosine kinase important for growth and survival functions. KIT mutations are described as being found with a frequency of 10–20% in both acrolentiginous and mucosal melanomas. KIT is a specific target of the kinase inhibitor Imatinib. This drug has shown good response in a small number of KIT-mutant melanoma patients.

Objectives: Fifteen cell cultures from 12 acrolentiginous and three mucosal melanomas were established. The status of KIT mutation and its correlation with *in vitro* proliferation of these cell cultures was assessed. A comparison between paraffin-derived DNA to cultured cell-DNA originating from the same tissue was performed for three patient samples. These three patients with mucosal melanoma were subsequently treated with Imatinib.

Results: Of fifteen cultures assessed for KIT mutation, twelve are wild-type and three show a mutation in V863L (exon 18), which has not previously been described for KIT. Of the three patient biopsies assessed two were positive for KIT mutations, L576P (exon 11) and K642E (exon 13). These two patients responded positively to subsequent Imatinib treatment, while the wild-type patient did not. Immunohistochemical staining for KIT showed it was expressed only in the mutant samples. Interestingly, the cell cultures derived from these KIT mutant biopsies were found to be wild-type for KIT. Imatinib-sensitivity proliferation assays are ongoing.

Conclusion: We confirm that KIT mutant patients respond better to Imatinib than KIT wild-type patients. However, it is striking that the cell cultures derived from these mutant-positive biopsies yield wild-type KIT. This suggests that the KIT mutation was not present in all cells within the lesion, but rather was heterogeneously distributed. This in turn indicates that KIT mutation is not an initial event in melanoma progression.

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Ambivalent effects of TNF alpha on apoptosis of normal vs. malignant human keratinocytes

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Aim: Several opposing results concerning the effect of TNF alpha on the homeostasis of keratinocytes have been published. In this project, the ambivalent effects of TNF alpha pretreatment followed by certain proapoptotic stimuli on normal in comparison with malignant keratinocytes were investigated.

Methods: Primary normal human keratinocytes (NHK) and the squamous cell carcinoma cell lines SCL-II were treated with TRAIL or 5FU with and without TNF alpha pretreatment. WST assay was used for cell viability estimation. Apoptosis was assayed by DNA fragmentation ELISA, whereas necrosis was measured by LDH-detecting colorimetric assay. Western blot (WB) analysis was performed for determination of caspase and Bcl-2 proteins expression. FACS analysis with TMRM staining was used for the evaluation of mitochondrial membrane potential.

Results: A cytoprotective effect of TNF alpha on normal human keratinocytes causing decreased apoptosis against both 5FU and TRAIL was observed. In contrast, TNF alpha pretreatment of malignant keratinocytes affected their viability and led to an enhancement of apoptosis after 5FU and TRAIL stimulation. In parallel, WB analysis revealed increased processing of caspase-8 and -3 after TNF alpha + TRAIL treatment in SCL-II cells, whereas processing of caspase 3 was reduced in NHK after TNF alpha + TRAIL as compared to TRAIL alone. Activation of the Bcl-2 proapoptotic protein Bid (processing) as well as expression of the antiapoptotic protein Mcl-1 was also involved in the enhancement of apoptosis in SCL-II cells after TRAIL treatment.

Conclusions: Stimulation of NHK with TNF alpha appears to activate a prosurvival pathway that protects the cells against both extrinsic (TRAIL) and intrinsic (5FU) induction of apoptosis. For malignant keratinocytes, in contrast, TNF alpha contributes to an enhancement of apoptosis induction by TRAIL and 5FU. This may be of clinical significance when anti-TNF alpha are applied.

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Effects of interferon alpha on the regulation of transporter proteins associated with antigen processing in antigen presenting cells and melanoma tissue

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IFN alpha is frequently used in adjuvant therapy of malignant melanoma but the complex molecular mechanisms of its antitumor effects are only partially understood. We examined the expression of transporter proteins associated with antigen processing (TAP) in peripheral blood mononuclear and tumor tissue of C57BL/6 mice injected s.c. with B16F1 melanoma cells receiving different regimen of systemic IFN alpha treatment. These *in vivo* studies proved a strong correlation between the enhanced TAP1 mRNA expression in PMBCs and tumor tissue and the decreased formation of subcutaneous melanoma metastasis in animals receiving adjuvant IFN alpha therapy.

Stimulatory effects of IFN alpha on TAP1 expression could also be demonstrated *in vitro* in human THP1, TUR and A375 melanoma cells. Compared to THP1 and TUR cells a 100fold higher concentration of IFN alpha had to be applied to A375 cells to detect a fourfold upregulation TAP1.

To evaluate whether the upregulatory effect of IFN alpha on TAP1 expression depends on the activation of STAT1, molecular studies in THP1 and TUR cells were performed. High concentrations up to 200 U/ml IFN alpha led to the activation of STAT1, STAT3, Erk1/2, p38 and Akt, whereas lower concentrations of 10 U/ml IFNalpha resulted only in the phosphorylation of STAT1. Pretreatment of THP1 cells with JAK Inhibitor I 45 min before stimulation with IFN alpha inhibited upregulation of TAP1/2. Similarly mutation of the STAT1 or the interferon regulatory factor binding site in the TAP1 promoter significantly decreased the expression of the luciferase reporter gene. For the IFN alpha-sensitive melanoma cell line A375 we obtained similar results. In addition, A375 cells transfected with wild type STAT1 revealed an enhanced, cells transfected with dominant-negative STAT1 revealed a decreased regulation of TAP1 mRNA expression after stimulation with IFN alpha.

These *in vivo* studies demonstrated an adjuvant effect of IFN alpha on the TAP1 expression in antigen presenting cells and melanoma tissue and *in vitro* experiments revealed that this effect is mainly mediated by STAT1 phosphorylation. Interestingly, significantly higher concentrations of IFN alpha have to be applied to stimulate TAP1 expression in melanoma cells compared to antigen presenting cells, which might in part explain the clinical advantage of a high dose therapy compared to low dose regimen.

P276 (V25)

Modification of the sphingosine kinase pathway as a novel therapeutic approach for melanoma

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Aims: Melanoma is highly resistant to conventional therapy. As sphingosine kinase type 1 (SK1) plays a critical role in determining the dynamic balance between the pro-apoptotic sphingolipid metabolite ceramide and the prosurvival S1P, we examined the role of the modification of the SK1 pathway as a novel therapeutic approach for melanoma.

Methods: Micro array, qPCR, Western blot, enzyme activity assay, cell proliferation assay (MTT), cell cycle flow analysis, migration assays, 3D spheroid melanoma model; multi-photon microscopy

As preclinical studies *in vitro* often poorly predict the outcome of clinical studies we use a novel cell culture model which better compares to the *in vivo* situation melanoma cells grown as 3D spheroids are implanted into collagen to mimic tumor architecture and microenvironment. Here we discuss the anti-melanoma activity of the modification of the SK1 pathway in our novel 3D spheroid models. The use of multi-photon microscopy allows real-time imaging of the interactions of melanoma cells with their microenvironment and their drug response.

Results: Micro array, qPCR and Western blot analysis showed the presence of SK1in 29 investigated melanoma cell lines, albeit at varying expression levels. Regardless of the expression level, enzyme activity assays proved the functionality of SK1. Modification of the SK1 pathway, using inhibitors of different stations within the pathway, led to significant melanoma growth inhibition at pharmacologically relevant doses. Interestingly, whereas dimethyl-sphingosine caused G1-phase cell cycle arrest and was cytostatic, sphingosine kinase inhibitor 2 (SKI-2) caused G2-phasecell cycle arrest. In the 3D model, the investigated inhibitors showed stage-dependent effects: radial and vertical growth phase melanomas showed earlier growth and invasion inhibition and more cell death than metastatic melanomas, as shown by real-time multi-photon microscopy analysis.

Conclusions: For the first time we show here that modification of the sphingosine kinase pathway in melanoma has cytostatic effects on melanoma in 2D and in 3Dculture. Ongoing studies *in vivo* will reveal the potential of these inhibitors as a novel therapeutic approach for melanoma.

P277 (V20)

Resistance to anti-angiogenic therapy is directed by vascular phenotype, vessel stabilization and maturation in malignant melanoma

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Angiogenesis is not only dependent on endothelial cell invasion and proliferation, it also requires pericyte coverage of vascular sprouts for stabilization of vascular walls. We hypothesized that the level of vessel maturation is critically involved in the response to anti-angiogenic therapies. To test this hypothesis we evaluated the vascular network in spontaneously developing melanomas of MT/ret transgenic mice after using PTK/ZK for anti-VEGF therapy but also analyzed human melanoma metastases taken at clinical relapse in patients undergoing adjuvant treatment using bevacizumab.

Melanoma of MT/ret transgenic mice revealed morphological analogy to human melanoma and express tyrosinase, tyrosinase-related protein-1 and gp-100, enzymes, regulating the quality and quantity of pigment production in melanocytes followed by the development of metastases in lymph nodes (100%), spleen (> 80%), lung and brain (> 10%). Immunohistological analyses on tumor vascular beds showed two different vascular phenotypes in this model independent on tumor volume nor location. One predominant type showed a high-angiogenic-active phenotype with mean microvessel density (MVD) of 250 mm^2 in contrast to a second type, characterized by only a few intratumoral vessels and mean MVD of 40 mm^2, subsequently described as low-angiogenic tumor. Fast growing highly-angiogenic-active tumors exhibt hypoxia-driven Angiopoietin-2 expression leading to immature intratumoral vascular network, basal lamina defects and loss of pericytes. In contrast, low-angiogenic-active tumor nodules displayed stabilized vessels, a slower growth kinetic and increased vessel lumina. Highly-vascularised tumors were characterized by significant tumor regression in the adjuvant – and remodelling of the tumor vascular bed in the advanced therapy setting. Interestingly, low-angiogenic-active tumors did not respond to anti-VEGF therapy using the small-molecule inhibitor PTK/ZK. Careful expression profiling analysis of laser microdissected tumor-associated ECs from both vascular beds revealed a significant decrease in expression of pro-angiogenic factors Ang-2 and VEGF-A and their receptors Tie2 and VEGFR-2 in VEGF-resistant MT/ret tumors.

In summary, we were able to show in both experimental settings, MT/ret-transgenic melanoma and human melanoma metastases, taken after adjuvant treatment using bevacizumab, that tumor vessels, resistant to anti-VEGF therapy, are characterized by enhanced vessel diameter ($P \leq 0.001$) and normalization of the vascular bed by coverage of mature pericytes, immunoreactivity for desmin, NG-2, PDGFR-β and the late stage maturity marker α-SMA in contrast to partly reduced pericyte coverage or lack of α-SMA-expressing pericytes on intratumoral microvessels vessels, sensitive to anti-VEGF therapy ($P \leq 0.001$). Our findings emphasize that the level of mural cell differentiation and stabilization of the vascular wall significantly contribute to the response towards anti-angiogenic therapy in melanoma.

P278

Mesenchymal stem cells exert an inhibitory effect on the activation of macrophages *in vitro* – Implications for the non-healing State of chronic venous leg ulcers

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There is accumulating evidence that activated macrophages play a major pathogenic role in the non-healing state of chronic venous leg ulcers (CVU). Previously we found highly activated macrophages to persist in high numbers with enhanced nitric oxide (NO) and pro-inflammatory TNF-α and IL-12 release perpetuating the inflammatory phase in CVU. In addition, severe dermoliposclerosis in the CVU leads to a progressive loss of the subcutaneous mesenchymal stem cell niche.

Autologous or allogeneic transfer of mesenchymal stem cells represents a promising treatment modality which may control persistent macrophage activation and rescue the regenerative capacity of chronic wounds. We therefore studied the role of MSCs on macrophage activation using co-culture of bone marrow-derived, plastic adherent MSCs (CD105+, CD73+, CD90+, CD45–, CD34–, CD14–, CD11b–, CD79α–, CD19–and HLA-DR-) and activated F4/80+ macrophages. Macrophages were effectively stimulated either with the combination of LPS and IFNγ or TNF-α and IFNγ, leading to enhanced release of NO, IL-12 and TNF-α. We found that co-culture of adherent MSCs with activated macrophages resulted in a significant reduction of NO, TNF-α and IL-12 release. Interestingly, the suppressive effect on NO release occurred in a biphasic manner strictly depending on the ratio between macrophages and MSCs. NO release was significantly decreased at co-culture ratios (MSCs: macrophages) ranging between 1:5×104 to 1:104 and between 1:50, 1:10 and 1:5, while no suppressive effect was detected at ratios of 1:2×103 or 1:1×103. Notably, MSCs significantly suppressed the IL-12 and TNF-α release only when co-cultured at MSCs:macrophage ratios ranging between 1:5×104 to 1:104, but not at higher ratios. A suppressive effect of MSCs on macrophage activation could be reproduced in 11 out of 16 studied bone-marrow derived MSCs lines and in 2 out of 2 adiposetissue-derived MSCs lines from different donors, suggesting that the inhibitory effect of MSCs on macrophage activation is independent of their tissue origin. Collectively, these data show that MSCs exert a suppressive – though not completely abolishing -effect on macrophage activation with reduction of pro-inflammatory cytokines. Thus, topical transfer of MSCs may qualify as a promising therapy in macrophage-dominated impaired wound healing. However, understanding the mechanisms underlying the MSC number-dependent biphasic effects needs to be clarified prior to the development of protocols for clinical applications.

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Induced pluripotency by transcription factors

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Induced pluripotent stem cells (iPS cells) can be generated from different cutaneous cell types through ectopic expression of the transcription factors Oct4 and Sox2, combined with Klf4 and c-Myc. iPS cells acquire all the features of embryonal stem cells including immortal growth and pluripotency, measured by their ability to differentiate into multiple cell types in teratomas and their contribution to germline-competent chimeras in mice.

A major limitation of reprogramming somatic cells into iPS cells is its low efficiency, which ranges in mouse and human cell types between 0.01–0.2%.

We show that cells with low endogenous p19Arf (encoded by the Ink4a/Arf locus) protein levels and immortal cells deficient in components of the Arf-Trp53 pathway yield iPS cell colonies with a faster kinetics and at a significantly higher efficiency, endowing almost every somatic cell with the potential to form iPS cells.

These results show that the acquisition of immortality is a crucial and rate-limiting step towards the establishment of a pluripotent state in somatic cells and underscore the similarities between induced pluripotency and tumorigenesis.

P280

Progressive decrease in number and change in niche preference of theABCB5+ mesenchymal stem cell subset in the skin during aging

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Recently, the expression of ABCB5, a novel P-glycoprotein of the ABC superfamily of active transporters, was found in a newly defined mesenchymal stem cell subpopulation in the skin. Even though its decrease in number and/or function may result in impaired regenerative capacity and aging, robust *in vivo* data are currently not available. Here we studied the cell surface expression pattern, the cell number and the specific localization of ABCB5-positive mesenchymal stem cells in human skin in young (0–20 years), middle aged (21–70 years) and old healthy individuals (>71 years). Dermal ABCB5+ cells showed variability for expression of the hematopoietic progenitor cell antigen CD34 or for CD133, indicating that they represent a distinct stem cell subset in human skin. In contrast, ABCB5+ cells expressed a panel of previously established mesenchymal stem cell markers, including CD29, CD90, CD59 and CD44. The specificity of the anti-ABCB5 mAb was confirmed using a competitive 16-mer peptide. Notably, a significant decline of the percentage of ABCB5+ cells was found in the old age group compared to the younger age groups ($P < 0.0001$), while the total number of resident cells in the dermis per high power field remained identical in all age groups. In a first attempt to understand the mechanisms underlying the ABCB5+ stem cell decline in old individuals, we studied the expression of γH2AX, a phosphorylated histone protein which detects DNA double strand breaks and initiates a DNA damage response with the induction of cell cycle inhibitors like p16. Preliminary data indicate that by contrast to age-dependent increase in γH2AX and p16 in dermal fibroblast, no consistent age-dependent increase in γH2AX and p16 was found in ABCB5+ mesenchymal stem cells. Interestingly, ABCB5+ mesenchymal stem cells were found in close association of CD31+ vessels in younger individuals, while this perivascular localisation was lost in the old age group. Collectively, a robust decrease in ABCB5+mesenchymal stem cells with changes in niche preference may contribute to a reduced regenerative capacity in skin aging.

P281 (V21)

Altered redox signalling in senescent human dermal fibroblasts

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The free radical theory of aging postulating increased concentrations of reactive oxygen species (ROS) to damage biomolecules and drive aging, is still controversially discussed. We here used a proteomic approach with 2D fluorescence difference gel electrophoresis (2DIGE) and mass spectrometry (MS) of protein spots differing in replicative senescent fibroblasts versus young fibroblasts to address the questions whether (1) imbalanced expression of antioxidant enzymes leads to redox imbalance, and if so (2) what is the identity of the predominant oxygen species which causes induction of ROS-dependent target genes responsible for connective tissue degradation in skin aging. Notably, we found manganese superoxide dismutase (SOD2) to be 13.2-fold overexpressed, while peroxiredoxins 2, 5, 6 which detoxify the SOD2-generated H2O2 were expressed only 1.6 to 1.8-fold in replicative senescent fibroblasts. This imbalance in H2O2 generation and its detoxification may result in redox imbalance. In fact, we confirmed SOD2 overexpression and increased activity using immunostaining, western blot analysis and a substrate-based activity assay. To further assess the anticipated redox imbalance in senescent fibroblasts, we loaded cells with DHE, a substrate which is ROS concentration-dependently converted to increasing fluorescence intensities. A significant two-fold increase in fluorescence intensity was found in senescent fibroblasts at a cumulative population doubling (CPD) of 70 as compared to young proliferating fibroblasts (CPD21) indicating an increase in ROS concentrations in senescent fibroblasts. To further specify the nature and identity of the prevailing ROS in senescent fibroblasts, we have used the adenoviral transduction of the H2O2 sensitive HyPer construct and the O2– specific MitoSox system. We found a significant increase in mitochondrial H2O2 levels and a decrease in O2– in senescent fibroblasts most likely due to enhanced SOD2 activity. Interestingly, SOD2 overexpressing replicative senescent fibroblasts revealed a3-fold overexpression and increase in the activity of interstitial collagenase/matrix metalloproteinase-1 (MMP-1) which is responsible for the collagen degradation during skin ageing. In order to study whether enhanced H2O2 concentrations are responsible for the AP-1 transcription factor-dependent MMP-1 induction, we have used transient transfection of the collagenase/MMP-1 promoter including the AP-1binding site (or a mutant AP-1 site) in front of the CAT reporter gene. A 4-fold higher transactivation of AP-1 was found in stably SOD2 overexpressing fibroblasts, while no transactivation occurred in the case of the AP-1 mutant construct. Collectively, w ehave found that enhanced SOD2 activity in replicative senescent fibroblasts via an unbalanced H2O2 overproduction and detoxification induces MMP-1, and this effect most likely is mediated by transactivation of AP-1. Targeting strategies for fibroblasts may hold considerable promise to re-establish redox balance and prevent or treat connective tissue degradation, a hallmark in skin ageing.

P282

A novel DNA damage pathway responsible for reduced ribosomal biogenesis and protein S6 specific translational control accelerates aging in connective tissue specific manganese superoxide dismutase deficient mice

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Previously, the hypothesis was forwarded that DNA damage inhibits the IGF-1 growth axis with substantial reallocation of energy from growth to DNA repair eventually leading to organ atrophy and aging. Here we have used the connective tissue specific manganese superoxide dismutase (MnSOD) deficient (mutant) mice which closely recapitulate intrinsic aging including skin atrophy, osteoporosis, kyphosis, thymus involution and reduced life span to understand the underlying mechanisms of the DNA damage response pathway. Using the adenoviral transduction of the H2O2 sensitive HyPer construct, we found a significant decrease in mitochondrial H2O2 levels in mutant fibroblasts, while an increase in O2– was observed in mutant mice using MitoSox analysis. Western blotting and immunohistochemistry suggested an increase in nitrosylated proteins and DNA double strand breaks, as detected by γH2AX. In fact, reduced serum IGF-1 concentrations and IGF-1 dependent signalling were found with reduced levels of phosphorylated (activated) AKT/PKB (Ser478) and the FRAP kinase as well as p70S6 kinase in mutant mice. Notably, the ribosomal S6 protein, a down stream target of the FRAP kinase which specifically controls the translation of mRNA transcripts containing an oligopyrimidine tract in their 5′ untranslated region (5′TOP), was less phosphorylated (activated) in mutant skin lysates. We found that the 5′TOP genes, like Cyclin D1, responsible for cell cycle progression and the major structural protein collagen type I, constituting> 30% of all other proteins, were significantly reduced on protein but not on mRNA level in dermal lysates, while the cell cycle inhibitor p16 was highly induced. Prelimenary data on feeding N-acetyl cysteine, a potent scavenger of O2–, indicate a partial rescue of this newly identified DNA damage response pathway. Collectively, these data for the first time show that pro-oxidant damage to the fibroblasts via double strand breaks, attenuated IGF-1 signalling results in the inhibition of ribosomal biogenesis with reduced translation of specific proteins responsible for the observed aging phenotype. These data unequivocally opens new avenues for the prevention and treatment of age-related diseases.

P283

CD44 regulates tight junction assembly and barrier function

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Upon barrier disturbance adult CD44 knock-out (KO) mice show delayed recovery of epidermal barrier function. This correlates with loss of apical polarization of lamellar body (LB) secretion. In simple epithelia tight junctions (TJ) are crucial for barrier function and regulate the polarized targeting of vesicles to either the apical orbaso lateral membrane. In addition, they are important for murine epidermal barrier function. Therefore we hypothesized that CD44 regulates TJ and associated cell polarity complexes, which in turn contributes to altered skin barrier function in CD44KO mice. We show a delay in embryonic barrier formation associated with a loss of apical LB localization in CD44 KO mice which correlates with alterations in localization and/or expression levels of TJ proteins as well as the polarity protein Par3. Simultaneously, activity of the small GTPase Rac1, a major regulator of TJ barrier function, was reduced. Importantly, normalization of barrier functions at E18.5 was paralleled by the recovery of these proteins. In primary keratinocytes, cell polarization and TJ barrier function was impaired in CD44 KO cells. Together, the results reveal an important function for CD44 in the assembly and function of TJ, suggesting their involvement in the skin barrier phenotype of CD44 KO mice.

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Cells of diabetic and non-diabetic origin show different susceptibility to connexin-43-mimetic peptide Gap27

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Connexin 43 (Cx43) is downregulated during early wound healing (WH) at wound margins. Several mouse models and the dysregulation of Cx43 in diabetic chronic wounds indicate that this downregulation is important for WH. Since the Cx43mimetic peptide (Gap27) has been shown to block Cx43 mediated intercellular communication and promote skin cell migration we were interested in its therapeutic potential to improve diabetic WH.

We investigated the effects of Gap27 on *ex vivo* wound healing models (WHM) and human keratinocytes and fibroblasts of diabetic and non-diabetic origin focussing on cell migration, proliferation, Cx43 expression, localization and phosphorylation as well as hemichannel function.

We have shown previously that Gap27 treatment of WHM results in decreased dye transfer, accelerated WH and elevated cell proliferation. Here we demonstrate that Gap27 decreases dye uptake through Cx hemichannels in cultured cells. Non-diabetic cell cultures treated with Gap27 after scratch wounding showed enhanced migration and proliferation. Surprisingly, diabetic cells were not susceptible to Gap27 during early passages. In late passages these cells showed a response to Gap27 treatment comparable to that of non-diabetic cells. Detailed analysis for the causes of the discrepancy between diabetic and non-diabetic cells revealed less dye uptake in diabetic cells through Cx hemichannels but exclude differences in Cx43expression, localization and Ser368-phosphorylation.

These data emphasize the importance of Cx43 in WH and suggest the application ofGap27 to be a beneficial therapeutic to accelerate normal but not necessarily diabetic WH. Additionally, we show that cells derived from diabetic patients exhibit a 'diabetic phenotype' that is lost after prolonged cultivation.

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Integration of Langerhans-like cells into a three-dimensional full-thickness skin model

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Langerhans cells (LCs) are immature dendritic cells residing suprabasally within the epidermis with Birbeck granules and cell surface receptor langerin (CD207) as typical markers. The CD34+ human acute myeloid leukemia cell line MUTZ-3 shows phenotypic characteristics of epidermal LCs including expression of langerin, CD1a and CCR6 after stimulation with a cytokine cocktail comprising GM-CSF, TGF-beta1and TNF-alpha. Furthermore, we show in differentiated MUTZ-3 cells (MUTZ-LC) the presence of Birbeck granules by transmission electron microscopy. The aim of the present study was to integrate MUTZ-LCs into a three-dimensional full thickness skin model consisting of keratinocytes and fibroblasts. First, primary human fibroblasts were seeded into a collagen matrix (Henkel AG & Co. KGaA, Düsseldorf). After 10 days primary human keratinocytes were seeded on top of the dermal equivalent. After 24 h differentiated MUTZ-LCs were seeded on top followed by a second keratinocyte seeding 24 h later. After further 24 h under submerse conditions the models were lifted up to the air liquid interface (ALI). Histological evaluation performed after 10–12 days featured a fully stratified epidermis with all characteristic epidermal strata. The identification of MUTZ-LC within the full-thickness skin model was performed by immunohistochemical stainings against langerin. Langerin-positive cells were detected suprabasally within the epidermis indicating that keratinocyte sand/or fibroblasts provide environmental conditions for long-time maintenance of MUTZ-LCs. This skin model provides a tool to further investigate the interaction between langerhans-like cells and other skin cells and particularly learn more about the cutaneous immune response.

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The serine protease inhibitor of Kazal-type (Spink) -6 is a selective inhibitor of Kallikreins-related peptidases

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Kallikreins-related peptidases (KLKs) are serine proteases, implicated in the desquamation process of the skin. Their activity is tightly controlled by epidermal protease inhibitors of the serine protease inhibitor of Kazal-type (Spink) family including the lympho-epithelial Kazal-type inhibitor (LEKTI), LEKTI-2 and the recently discovered Spink6.

Herein we investigated the inhibition of most of the 15 KLKs members by Spink6 using colorimetric *in vitro* assays. Spink6 inhibited efficiently KLK4, 5, 6, 12, 13 and 14 with a Ki of around 1 *n*M. KLK7 was only inhibited slightly with a calculated Ki of 1 μM. On the other hand, KLK3, 8 and 11 were not inhibited by Spink6. Interestingly, no other serine proteases tested, including neutrophil elastase, tryptase, trypsin, mast cell chymase, thrombin, chymotrypsin, plasmin, cathepsin G and matriptase, were inhibited by Spink6.

Our data suggest that Spink6 is a selective inhibitor of some distinct members of the KLK family and might have therapeutic potential to circumvent elevated KLK levels.

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Collagen lattice contraction is enhanced by overexpression of manganese superoxide dismutase in human dermal fibroblasts - implications for fibrotic and fibro proliferative disorders

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Tissue homeostasis of the skin and different organs depend on regulated cell-matrix interactions and, if disturbed, may lead to fibrotic or fibro proliferative conditions. We have studied fibroblasts stably overexpressing manganese superoxide dismutase (MnSOD) with a defined capacity for the removal of superoxide anions and concomitant accumulation of hydrogen peroxide to evaluate the role of enhanced MnSOD activity on the dynamics of cell-matrix interactions in the three-dimensional collagen lattice contraction assay. MnSOD overexpressing fibroblast populated collagen lattices revealed a significantly enhanced contraction compared to collagen lattices populated with vector control cells or with MnSOD deficient fibroblasts. The enhanced collagen lattice contraction was in part due to an increase in activeTGF-β1 and the accumulation of H2O2 in MnSOD overexpressing fibroblasts populated collagen lattices as determined by adenoviral transduction of the H2O2 sensitive HyPer construct. Inhibition of TGF-β1 signalling by the ALK4,5,7kinases' inhibitor SB431542 at least partly inhibited the enhanced collagen lattice contraction of MnSOD overexpressing fibroblasts populated lattices. In addition, supplementation of vector control fibroblast populated collagen lattices with recombinant TGF-β1 concentration dependently enhanced the collagen lattice contraction. In the presence of the antioxidant Ebselen, a mimic of H2O2 and other hydroperoxides/peroxynitrite-detoxifying glutathione peroxidase, collagen lattice contraction and the activation of TGF-β1 were significantly reduced in collagen lattices populated with MnSOD overexpressing fibroblasts. Collectively, these data suggest that H2O2 or other hydroperoxides or peroxynitrite or a combination there of may function as important second messengers in collagen lattice contraction and act at least in part via TGF-β1 activation. It remains to be elucidated whether redox imbalance is responsible for the observed increase in active TGF-β1 concentrations and contractures in other fibroproliferative disorders.

P288

Human Thy-1 (CD 90) is a critical molecule for granulocyte emigration during inflammation

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The adhesion of inflammatory cells to endothelial cells plays a central role in inflammation. The interaction between Mac-1 (CD11b/CD18) on inflammatory cells and ICAM-1 on endothelial cells is a predominant adhesion event mediating the firm adhesion of leukocytes to endothelial cells at sites of inflammation. But, the presence of additional leukocyte-endothelium adhesion pathways mediated by interaction of Mac-1 with a ligand distinct from ICAM-1 was suggested in different studies.

Recently, we characterized the human glycoprotein Thy-1 (CD90) as an activation-associated molecule on human dermal microvascular endothelial cells. Resting microvascular endothelial cells did not express human Thy-1. In contrast, stimulation of microvascular endothelial cells with TNF-α induced expression of Thy-1. Accordingly, Thy-1 is only detectable on endothelial cells in inflamed skin. Functional assays demonstrated that the human Thy-1 acts as an adhesion molecule for the binding of neutrophil and eosinophil granulocytes to activated endothelial cells. We identified Mac-1 as receptor for Thy-1 on granulocytes and thus demonstrated that human Thy-1 essentially contributes to leukocyte recruitment to sites of inflammation. These achievements provide a new pathway for adhesion and transmigration of granulocytes. The interaction of granulocytes to Thy-1 provided not only the mechanical support but furthermore triggered granulocyte effector functions such as the secretion of matrix metalloproteinases (MMP-9) and chemotactic factors (CXCL8).

Finally, we confirmed the importance of Thy-1 for the control of emigration of granulocytes during inflammation in an experimental induced inflammation model. Here, the emigration of eosinophils was significantly reduced in Thy-1 deficient mice compared to wild type mice.

The data presented, prove that the human Thy-1 is an essential endothelial cellr eceptor for the leukocyte integrin Mac-1, that contributes to granulocyte recruitment to sites of inflammation.

P289

Insulin signaling contributes to the maintenance of epidermal homeostasis which is disturbed in psoriatic keratinocytes due to inflammation induced insulin resistance

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Besides insulin's well known effects on classic metabolic tissues, such as liver, muscle and adipose tissue, several reports suggest a role in non-metabolic tissues such as the skin. Psoriasis patients often display signs of insulin resistance. Therefore we examined the role of insulin signaling in the skin and whether a disturbed insulin response contributes to the development of the psoriatic phenotype in the skin.

Using HaCaT cells as well as primary human keratinocytes we could show that insulin activates PI3-Kinase as well as MAPK dependent pathways. We found that insulin induces expression of cytokeratin 1 and 10, markers of terminal differentiation, suggesting that insulin drives differentiation of healthy keratinocytes. Measuring proliferation as well as differentiation markers in a new combined FACS assay, we discovered that insulin regulates the equilibrium between proliferation, differentiation and apoptosis that is crucial to maintain a healthy epidermis.

We next examined the situation in psoriasis, where patients show systemically and locally (dermal) elevated levels of inflammatory cytokines, which are known to confer insulin resistance in metabolically active tissues. Using immunohistology we foundt hat components of the insulin signal pathway show altered activation patters in psoriatic lesions compared to non-lesional skin in the same patients. Therefore we analyzed whether this effect is also mediated via mechanisms of insulin resistance. Treating keratinocytes *in vitro* with inflammatory cytokines, namely IL-1beta, IL-12and IL-22, severely interfered with insulin signal transduction. In addition keratinocytes differentiation is abnormal and shifted towards hyperproliferation.

In summary our results indicate that psoriasis associated cytokines severely interfere with insulin signal transduction and therefore contribute to the development of the pathology.

Thus, controlling correct insulin signaling in the skin might represent a novel anti-psoriatic strategy.

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Time-lapse intravital imaging in long bones reveals altered cellular dynamics and endosteal location of aged early hematopoietic progenitor cells: implications for stem cell aging

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One hypothesis for aging in tissues with a high cell turnover like skin, intestine and the hematopoietic system is that tissue specific stem cell aging leads to perturbed homeostasis which presents as aging. We use as a model system to study stem cell aging the hematopoietic system. We recently hypothesized that one underlying cause of altered hematopoiesis in aging might be due to altered interactions of aged stem cells with the microenvironment/niche. We developed time-lapse intra-vital2-photon microscopy (MP-IVM) for murine long bones and novel image analysis algorithms to quantify the dynamics of young and aged hematopoietic cells inside the marrow of long bones of mice *in vivo*.

As anticipated from a model in which primitive cells locate closer to a stem cell niche called endosteum compared to more differentiated cells our analysis revealed that11% of transplanted early hematopoietic progenitor cells (eHPCs) had direct contact to the endosteum in the tibia whereas only 4% of eHPCs were in direct contact with the endosteum. In addition, eHPCs resided on average closer to the endosteum compared to progenitor cells (11 vs 19 μm). To mathematically quantify the extent of cell protrusion movement over time, we developed algorithms to calculate both the change in volume and the surface area over time of the observed cells based on the data obtained from the MP-IVM. Interestingly, aged eHPCs cells presented with significantly increased protrusion movements compared to young eHPCs as indicated a significantly ($P < 10.6$, F-test) increased average variance in cell volume over time. Aged eHPCs cells were located at sites more distant from the endosteum (average distance from the endosteum of 18.1 μm for aged compared to10.7 μm for young) with aged eHPCs residing as far as 40 μm away from the endosteum. Aged stem cells also presented with altered cellular polarity. These data might thus imply that aged eHPCs reside in distinct niches and/or interact with stroma cells with a distinct dynamic. In general, these data support a role of altered stem cell dynamics and altered cell polarity and thus altered niche biology in mechanisms of mammalian stem cell aging.

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Expression of mouse Spink12 in skin – a putative orthologue to humanSpink9

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Kallikreins-related peptidases (KLKs) contribute to the epidermal barrier function. Their activity is controlled by epidermal protease inhibitors. Recently we could identify a new KLK5-selective protease inhibitor lympho-epithial kazal-type related inhibitor (LEKTI)-2 encoded by its gene serine protease inhibitor of Kazal type (Spink)9 in human plantar and palmar skin. While LEKTI function has been extensively studied in the context of Netherton syndrome, the role of LEKTI-2 remains elusive. To learn more about LEKTI-2 functions in a mouse model system, we first of all tried to identify the mouse orthologue of human Spink9.

We identified mSpink12 as a putative orthologue to hSpink9 by phylogenetic analysis. mSpink12 exhibited 42% identity and 64% homology to hSpink9, which was the highest homology among all mSpink-members. Real time PCR analyses revealed mSpink12 mRNA expression in murine skin and epididymis. We expressed Spink12 recombinantly in E. coli and purified the protein by Ni-affinity column and reversed phase chromatography. The predicted mass was confirmed by electrospray ionisation mass spectrometry. Preliminary protease inhibition tests showed no decrease of the activity of human KLK5, which is inhibited by the hSpink9/LEKTI-2, and other serine proteases tested including trypsin, thrombin chymotrypsin, chymaseand plasmin.

In conclusion, we suggest that mSpink12 might be the mouse orthologue to human Spink9. Further investigations are needed to identify its function in murine skin.

P292 (V22)

Peroxisome proliferator-activated receptors regulate the expression of cathepsin B in human endothelial cells

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Malignant tumor cells recruit vasculature and stromal cells through production and secretion of stimulatory growth factors and cytokines to activate their local host tumor microenvironment. In this context, the reorganization of the extracelluar matrix is an important tumor initiated process. In the last years a group of lysosomal proteases, the cathepsins, especially Cathepsin B and L, were described to be crucial in the process of metastasis and angiogenesis. Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors, mainly implicated in the regulation of lipid and glucose homeostasis. In addition, PPAR agonists have also shown to function as anti-inflammatory and anti-angiogenic molecules.

We therefore explored the effect of PPARalpha and PPARdelta agonists on Cathepsin B expression by endothelial cells. Both inhibited the endothelial Cathepsin B protein expression in a time and dose dependent manner. In contrast, the lysosomal proteases, Cathepsin D and Cathepsin L, were not altered by PPARalpha or PPARdelta agonist treatment. We further investigated whether the inhibition in Cathepsin B protein synthesis by PPARalpha and PPARdelta ligands is mediated by changes in the mRNA expression level. Interestingly, treatment with PPARalpha agonist considerably suppressed Cathepsin B mRNA accumulation, whereas PPARdelta ligands failed to change the mRNA expression levels. Analysis of 5-deletional Cathepsin B promoter-based constructs revealed, that PPARalpha ligands mediate their inhibitory effects on Cathepsin B expression through an E-box-binding site in close proximity to the transcriptional start site. EMSA analysis demonstrated that PPARalpha activators reduce the binding of the heterodimerUSF1/USF2 at the E-box located between the base pairs -16 and +17 at the Cathepsin B promoter sequence. Hence, modulation of the rate of gene transcription represents the preferable molecular mechanism by which PPARalpha ligands inhibit Cathepsin B expression. In further analysis we could demonstrate that PPARdelta agonists conveyed their suppressive effects by a shortened Cathpsin B protein half-life. As the translational efficiency of Cathepsin B mRNA can depend in part on their 5′- and 3′-untranslated regions (UTRs), we hypothesized that the inhibitory effect of PPARdelta ligands on Cathepsin B expression might be mediated via an UTR dependent mechanism. First transcriptional activation studies with Cathepsin B UTR reporter gene constructs demonstrated that PPARd ligands do not convey their inhibitory effects on Cathepsin B expression by the 5′UTR. Further 3′UTR reporter gene studies are now under investigation.

In conclusion, our data identify for the first time endothelial Cathepsin B expression as a novel target for PPARalpha and PPARdelta agonist. As antivascular effects of PPARs are increasingly appreciated, enhanced insights in their modes of action are critical to define more optimal combinations with additional classes of therapeutic compounds.

P293

Trichohyalin-like 1, a novel member of the S100 fused-type protein family

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Genes of the S100 fused-type protein (SFTP) family, clustered within the epidermal differentiation complex on human chromosome 1q21.3, encode essential components that maintain epithelial homeostasis and barrier functions. We have recently identified hornerin and filaggrin-2 as two members of this family which are important components of healthy epidermis. Here we report the identification of trichohyalin like-1 (THHL1), a novel member of the SFTP family, which is mainly present in the hair. THHL1 encodes a glutamine- and lysine-rich protein of approximately 99 kDa, which shares common structural features with other SFTP members, in particular trichohyalin. THHL1 transcripts were detected at trace levels both in the skin and in cultured primary keratinocytes, while the protein could not be detected by Western blot analysis. Immunohistochemical analysis revealed that THHL1 is localized in the inner root sheath of hair follicles. These data suggest that THHL1 and trichohyalin might have overlapping and perhaps synergistic roles in the formation of the hair shaft.

P294 (V34)

Mast cell proliferation and differentiation is promoted by adhesion to fibroblasts via both Kit – dependent and – independent pathways

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The mechanisms of mast cell (MC) homeostasis in peripheral tissues are largely unknown and may involve proliferation, apoptosis, migration, and differentiation of MC precursors. Bone marrow derived cultured MCs (BMCMCs) exhibit increased proliferation and a change of their phenotype towards the connective tissue type MCs (CTMCs) when cocultured with fibroblasts (Fbs). The aim of our study is to evaluate the influence of Fbs on MC homeostasis and function. Interestingly, we observed that BMCMCs exhibited a strong adhesion to Swiss albino 3T3 Fbs. Thus, we analysed the regulation of proliferation and differentiation of BMCMCs with focus on the impact of this directed adhesion to Fbs. Surprisingly, the proliferation of BMCMCs was markedly increased only if MCs underwent a direct cell-to-cell contact to Fbs. Furthermore, the increase of BMCMC histamine content and mast cell protease 4 (MCPT4) mRNA expression was dependent on direct MC adhesion to Fb as well. Most notably, Kit-deficient MCs also showed a marked albeit lesser increase in proliferation and MCPT4 expression when cocultured with Fbs, which suggests an SCF/Kit-independent pathway for the modulation of MC numbers and phenotype by Fbs. However, the Kit-deficient BMCMCs showed no differences in their adhesion to Fbs compared to wild type BMCMCs. Furthermore, we found that vascular cell adhesion molecule 1 (VCAM-1) is involved in the adhesion to Fbs of both wildtype and Kit-deficient BMCMCs. Thus, our data show that BMCMC proliferation and differentiation towards CTMCs induced by Fbs is dependent on cell adhesion, but mediated by at least two separate pathways, one kit-dependent and the other kit-independent. The identification of adhesion molecules and membrane bound receptors other than Kit that induce MC proliferation and differentiation may provide interesting therapeutic targets for MC-driven diseases.

P295

The retinoblastoma tumor suppressor protein in aging and immunity

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The retinoblastoma tumor suppressor protein (RB) plays important roles in the control of the cell cycle, DNA-damage checkpoint, differentiation and apoptosis. Positive as well as inhibitory signals are integrated into the phosphorylation of the RB protein to regulate the G1 to S-phase progression of the cell cycle. The consequences of loss of RB on immune cell function *in vivo* are still not clear and have been controversially discussed. Using Cre-enzyme expression driven by the hematopoietic specificVav1-promotor, we generated mice that are constitutively deficient in RB (Vav1Rb–/–animals) in all hematopoietic cells. We already demonstrated that Vav1Rb–/–mice showed anemia with an increased number of reticulocytes in PB, consistent with a published role of RB in erythroid differentiation. Upon transplantation into NOD/SCID animals or upon competitive transplantation into C57BL/6.CD45.1 animals, hematopoietic stem cells from Vav1Rb–/–mice contributed 4 to 6-fold less to hematopoiesis. We conclude that upon transplantation/stress, hematopoietic stem cells from Vav1Rb–/–animals are impaired in their self-renewal function. The frequency of myeloid cells in BM was increased up to 70% compared to 40% in control animals, whereas the frequency of B220 positive B-lymphoid cells was almost10-fold reduced, without affecting the T-lymphoid compartment. These data imply a role for the RB protein in the development and/or the long-term function of immune cells. We are currently investigating immune cell function in animals deficient for RBin all hematopoietic cells. Novel lifespan studies revealed a significantly reduced lifespan for animals deficient in Rb in hematopoietic cells, with a maximum lifespan of less than 60 weeks. This reduction of lifespan though is not linked to leukemia development. We propose altered immune cell function as the driving force in lifespan reduction in Vav1Rb–/–animals. In summary, loss of RB results in context/localization dependent phenotypes in the hematopoietic hierarchy, influencing stem and progenitor cells differentiation abilities towards immune cells and possibly their function.

P296 (V13)

Evaluation of the role of autophagy in epidermal keratinocytes

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Autophagy is a cellular 'self-eating' process whereby organelles or parts of the cytosol are degraded. Epidermal keratinocytes undergo extensive intracellular remodelling during cornification, involving the complete degradation of nuclei and mitochondria, and restructuring of the cytoskeletal proteins. Since terminal differentiation of epidermal keratinocytes is incompletely understood at the molecular level, we investigated the potential role of autophagy in this process. ATG7, an essential regulator of classical autophagy, was inactivated by use of the cre/LoxP system specifically in the epidermis. Ablation of ATG7 expression was confirmed by RT-PCR and Western blot analysis. Loss of ATG7 suppressed the formation of the autophagosome-bound form of LC3, LC3-II, upon treatment with rapamycin together with protease inhibitors, confirming the critical role of ATG7 in keratinocyte autophagy. ATG7-flox K14-Cre mutant mice were viable, young mice appeared grossly normal, and neonates did not show skin barrier defects as judged by toluidine blue dye exclusion. Similarly, adult mutant mice displayed trans-epidermal water loss similar to controls. Histological and electron microscopic examination of skin revealed that the absence of ATG7 in keratinocytes did not prevent the formation of an orthokeratotic stratum corneum *in vivo*. However, sebaceous glands of mutant mice consistently appeared larger, hair shafts contained increased amounts of sebum, there was a tendency for mitochondrial DNA in hair to be increased, and an increase in loricrin expression. From about 12 months of age, the majority of mutant mice, but only few controls, suffered from hair loss on the trunk, despite the presence of anagen hair follicles. Those mutant mice with the most severe hair loss showed an increased thickness of the epidermis, increased loricrin expression and an accumulation of loricrin negative keratohyaline granules in the stratum granulosum and infundibulum of the hair shaft. Taken together, our data demonstrate that ATG7-dependent autophagy is not essential for cornification of epidermal keratinocytes, but plays an aging-associated role in epidermal homeostasis and hair growth.

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2-Photon-imaging of skin: single versus multi-beam fluorescence lifetime imaging detection-systems

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Two-photon-microscopy is a popular and powerful method for imaging of tissues and cells. Through its near infrared excitation in a very small volume of synthetic, intrinsic and targeted fluorophores in the specimen, phototoxicity and photobleaching are reduced to a minimum. Through FLIM (fluorescence lifetime imaging microscopy), in homogeneous dye distribution and emission-wavelength limitations may be circumvented. Yet, all 2-photon methodologies are inherently 'dark', the low probability of the 2-photon effect therefore requires sensitive detection systems.

Most common FLIM-detectors are used with a single-beam point-scanning and PMT (photomultiplier tube) detection. We here compare a multiplexed setup, a 16-channeltime-correlated single photon counter (TCSPC), which correlates single photon-events with an excitation laser pulse in single beam mode at a sampling rate of max. 78 Mhz. The alternate approach is a time-gated CCD-camera (Picostar), with a multi-focal beam-system of up to 64 single foci for accelerated scanning.

Both systems were used in *ex vivo* porcine skin models stained with fluorescein. We detected minor advantages for the TCSPC system in resolution; this system measures time-parallel events, is easily saturated but insensitive to scattered light. The Picostar system proved advantageous at high signal intensities as it measures locally parallel, although with scattered light sensitivity.

For TCSPC, we found an approximate point-spread-function (PSF) of 350 *n*m in x-y direction, which deteriorated by a factor of 3 for Picostar. In z-direction both systems reached the theoretical maximum of the lense in use, here 1.3 μm. The minimum penetration depth for TCSPC reached 300, for Picostar 200 μm; acquisition rates under our current experimental conditions did not vary greatly.

We are currently fine-tuning the detection process to maximize data acquisition and speed for 3–5 dimensional data-stacks from skin samples.

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Two chronobiological systems meet: Genes, proteins and microRNAs central to circadian rhythm control are differentially regulated during cyclic human hair follicle cycling

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Twenty-four hour circadian rhythms are controlled by differential clock gene activity. The human hair cycle is another fascinating chronobiological system with a unique periodicity spanning months to years, but has widely been ignored by main stream chronobiology research. Following the recent discovery that selected circadian clock genes are also involved in the control of murine hair follicle cycling, we have explored whether clock genes and key regulators of clock gene expression, microRNAs, are differentially expressed in human hair follicles (HFs) during different hair cycle stages.

Human scalp HFs were micro-dissected and organ-cultured in serum-free medium. The miRNA, mRNA and protein expression of morphologically identified anagen VI orcatagen HFs was characterized and compared using TaqMAN® miRNA arrays, Q-PCR and/or immunohistology.

Compared to anagen VI HFs, catagen HFs showed increased relative gene expression levels of Clock, Cry2 ($P < 0.05$) and Period1 (not significant at 0.05 level) and decreased expression of Cry1 ($P < 0.01$). PERIOD1 protein expression was significantly increased in human anagen VI compared to catagen HFs, with its expression localised to the hair matrix and outer root sheath layer of the HF. Interestingly, the key hypothalamic neuroendocrine regulator of clock gene activity, TRH, which we have recently discovered to potently stimulate human HF growth *in vitro*, also significantly up-regulated the intra-follicular transcription of Bmal1 and Cry1 ($P < 0.01$). Twenty-seven miRNA species associated with suppressing the translation of selected clock gene mRNA transcripts showed marked differential regulation between anagen and catagen HFs

These results suggest that the human 'hair cycle clock' utilizes key molecular players of circadian chronobiology. This invites novel strategies for the manipulation of human hair growth via the manipulation of clock gene expression and activity.

P299

A novel player in the regulation of human pigmentation: Golgi-located N-acetylglucosaminyl-1-phosphotransferase (GNPTA) is involved in POMC processing and melanin production *in situ* and *in vitro*

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The phosphorylation of mannose residues in lysosomal enzymes, essential forendosomal/lysosomal targeting, is catalyzed by a specific N-acetylglucosaminyl1-phosphotransferase, composed of three subunits, alpha 2beta 2gamma 2 that are products of two seperate genes (GNPTA and GNPTG). Defects in the phosphotransferase genes prevent (GNPTA) or impair (GNPTG) the formation of mannose 6-phosphate recognition residues followed by missorting of lysosomal enzymes and intracellular reduced levels of lysosomal enzyme activities characteristic for mucolipidosis type II (ML II, I-cell disease) or type III (ML III, pseudo Hurler-polydystrophy). Beside the severe metabolic and skeletal phenotype this patients show a thickening of the skin and bright-blonde hairs. Until now, GNPTA has only been known to play a role in the above mentioned endosomal/lysosomal pathway. Here, we provide evidence that the phosphotransferase is also involved inpro-opiomelanocortin (POMC) processing in normal human hair follicle epithelium. GNPTA knock-down by lipofectamine mediated siRNA transfection in organ-cultured adult human scalp hair follicles substantially reduced POMC mRNA and protein expression and strongly reduced intrafollicular melanin production (as shown by Masson-Fontana histochemistry and melanin content/cell). GNPTA knock-down also significantly inhibited melanin production, reduces the gp100 mRNA and protein level in isolated, cultured primary human hair follicle melanocytes. This effect was not just due to a non-specific blockade of the M6P-receptor-mediated transport of proteins to the endosomal/lysosomal compartment, because incubation with NH4Cl did not change POMC gene activity, but resulted in a reduced intracellular ACTH protein level in hair follicle melanocytes. In summary, our study identifies this golgi-located phosphotransferase as a novel player in the regulation of human pigmentation, namely in POMC processing and melanin production within human hair follicles and their melanocytes.

P300

The role of kindlin-2 in fibroblast functions: implications for wound healing

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Recent advances in research on kindlin-2 underlined its contribution to integrin activation in developmental processes. However, its role in the homeostasis of adult tissues has remained unexplored. In contrast to kindlin-1, a key protein in Kindler syndrome, which is restrictively expressed in epithelial cells, kindlin-2 is expressed at high levels in all major skin cell types - keratinocytes, fibroblasts and melanocytes. Here we assessed physiological functions of kindlin-2 in adult human skin and in primary dermal fibroblasts *in vitro*. siRNA technology, which was employed to suppress kindlin-2 expression to obtain indirect information, helped uncover new functions for this kindlin. The experiments demonstrated that kindlin-2 is essential for proliferation, spreading and directed migration of dermal fibroblasts, and moderately contributes to their adhesion. It is also involved in cell-matrix interactions, in transmitting mechanical cues between cells and their microenvironment and in the regulation of matrix deposition. These functions are highly relevant in tissue repair, and subsequently we identified kindlin-2 as a contributor to integrin and PDGF / FGF signaling networks involved in the middle phases of repair in human skin wounds. These data shed new light onto physiological functions of kindlin-2 in inside-out and outside-in signaling in dermal fibroblasts and disclosed its hitherto unknown role in wound healing.

P301

Modulation of mast cell biology by thrombin via proteinase-activated receptors (PARs)

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The serine protease thrombin, a mayor player in the coagulation cascade, is cleaved from prothrombin by factor X in every injury situation and acts by proteolytic cleavage of proteinase-activated receptors (PARs). Lately, it was shown that thrombin has proinflammatory actions on different cell types via activating PARs. Additionally, recent studies demonstrate that the well known thrombin receptor PAR1 is expressed on mast cells (MCs). MCs, which are highly inflammatory cells, are located in the skin and around blood vessels and critically contribute to skin inflammation. This is why we investigated the impact of thrombin on MC function. To this end, we used two different models of *in vitro* cultured murine MCs, immature bone marrow derived mast cells (BMCMCs) and peritoneal cultured MCs (PCMCs) as mature connective tissue type MCs. We found that both murine MC subtypes expressed mRNA for all thrombin receptors e.g. PAR 1, 3, and 4. In addition MCs were activated by thrombin in a dose-dependent manner as assessed by histamine and β-hexosaminidase release assays. Using a PAR1 activating peptide (PAR1-ap) we confirmed the evidence of MC activation by thrombin via PAR1. Furthermore we found that stimulation of MCs with thrombin or PAR1-ap results in the release of MCP-1 in a dose- and time-dependent manner. The intra cutaneous injection of thrombin into the ears ofC57BL/6 mice resulted in a significant immediate inflammatory skin reaction associated with a prominent ear swelling. Surprisingly, this ear swelling was even more pronounced in MC-deficient C57BL/6 KitW-sh/W-sh mice. Thus, our data show that thrombin is a potent MC activator that can induce MC degranulation and chemokine production via PARs, specifically via PAR1 *ex vivo*. Moreover, immediate inflammatory skin reaction to thrombin may be controlled by cutaneous MC. Taken together, PAR-activated mast cells may be important modulators of inflammatory responses to thrombin and potential targets for treating injury-associated inflammation.

P302

Expression Pattern and Characterization of Murine Filaggrin-2

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The mammalian skin functions as physical, chemical, and biological barrier organ of the body. It provides protection against water loss, radiation, and invading microorganisms. The barrier function is mainly due to the properties of the stratum corneum, the outmost and most differentiated cell layer of the epidermis. During terminal differentiation of keratinocytes that eventually lead to the formation of the stratum corneum, one family of proteins that are increasingly expressed is the 'S100 Fused Type Protein' (SFTP) family. Recently we have identified the human profilaggrin-related protein filaggrin-2 (FLG2) as a member of the human SFTPs. The murine FLG2 is similar to all members of this family comprised of three exons of which the first is non-coding. The full length mRNA is approximately 7.75 kb and codes for a 2362 amino acid long protein. Two EF-hand domains are located at the N-terminus that is the characteristic for this protein family. The following sequence can be divided into a spacer sequence that seems to be related to the profilaggrin B-domain and 14 repeat domains of 73–80 amino acids in length. To address the question if murine FLG2 shows an expression pattern similar to the human protein different tissues were first analyzed by RT-PCR with intron-spanning primers. An antibody generated against the N-terminal region of human FLG2 showed cross reactivity to the mouse FLG2 due to high identity to the murine N-terminal sequence. This antibody was used for immunohistochemical and Western Blot analyses. The murine FLG2 shows comparable expression patterns on mRNA as well as on protein level in the upper cell layers of the skin as detected for the human protein. In addition both mRNA and protein expression was observed in paw pads, nose, tongue, esophagus, and forestomach. A stimulation with elevatedCaCl2-concentration showed an upregulation of FLG2 mRNA in cultured primary murine keratinocytes whereas barrier disruption had no effect on mRNA or on protein expression level. Western Blot analyses of skin extracts showed immunoreactive bands only when treated with SDS. The presence of multiple bands lower than the expected full length protein indicate a possible processing of the FLG2 protein as it is already known for profilaggrin. Murine FLG2 shows an mRNA and protein expression similar to the human FLG2 and seems to be expressed in a differentiation-dependent manner as pointed out by RT-PCR, immunohistochemistry, and Western Blot analyses. Therefore it may have important functions in cornification that perhaps reinforce or supplement the functions of profilaggrin during this process of terminal differentiation.

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KGF-dependent cell signaling is regulated by ADAM10-mediated FGFR2b cleavage

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Fibroblast growth factor receptor 2b (FGFR2b) is a member of the tyrosin kinase receptor family and essential for embryogenesis. The epithelial isoform of theFGFR2, also named Keratinocyte growth factor receptor (KGFR), plays a key role in epithelial cell proliferation and differentiation. Fibroblast growth factor 7 (FGF7/KGF) is a specific ligand for FGFR2b. The loss of FGFR2b or defects in the ligand binding region results in different diseases like Crouzon-Syndrome or Jackson-Weiss-Syndrome. Protein ectodomain shedding is a critical regulator of many membrane proteins, including epidermal growth factor receptor ligands like TGF-alpha or other cell surface receptors like vascular endothelial growth factor receptor (VEGFR).

In this study we analysed whether FGFR2b might also be a substrate for ectodomain proteolysis and set out to identify the responsible protease. Using inhibitor studies, overexpression and RNA interference experiments we found that the disintegrin-like metalloprotease ADAM10 is critically involved in FGFR2b shedding. Moreover the cleavage seems to be induced by ligand binding and several signaling pathways. Our results indicate that ADAM10 mediated cleavage regulates FGFR2b signaling function in keratinocytes.

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Cell-based therapy of inherited epidermolysis bullosa by bone marrow-derived hematopoietic stem cells

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Desmoglein 3 (Dsg3) is a desmosomal transmembrane protein required for regular cell-cell adhesion and cellular function of basal keratinocytes in stratified squamous epithelia. Genetically engineered mice with a targeted disruption of the Dsg3 gene(Dsg3–/–) develop a PV (Pemphigus vulgaris)-like phenotype, characterized by mucosal erosions, a typical blistering histology, cyclic telogen hair loss and the disability to gain weight in comparison to heterozygous Dsg3+/– mice. Therefore, Dsg3 knockout mice represent an excellent animal model to study severe skin fragility disorders, like inherited epidermolysis bullosa (EB) in humans. So far, there is no treatment for EB available. We have established an experimental cell-based therapy to examine the ability of bone marrow (BM)-derived hematopoietic stem cells (HSCs) to repair epithelial defects of Dsg3–/– mice and to restore functionalDsg3-expression after systemic application. After stem cell transplantation (SCT) of BM-derived HSCs from transgenic mice, expressing the enhanced green fluorescent protein (EGFP), Dsg3–/– mice were able to continuously gain weight, similar to theirDsg3+/– control mice. Immunofluorescence microscopy revealed that EGFP+ positive BM-derived HSCs selectively migrated into epithelial defects only of Dsg3–/– mice, and restored Dsg3-expression and epithelial structure by K5+ basal epithelial cells. Surprisingly up to 56% of all epithelial cells in Dsg3–/– mice were EGFP+ after SCT, compared to only 6% of EGFP+ epithelial cells in transplanted Dsg3+/– control mice. In contrast, both, the Dsg3–/– and the Dsg3+/– mice, showed a similiar engraftment of bone-marrow with more than 50% of EGFP+ positive cells. Ultrastructually theDsg3–/– mice re-established normal desmosomal architecture after transplantation. Our results indicate that epithelial defects of Dsg3–/– mice constitute unique microenvironmental niche for BM-derived HSCs to migrate into, and to perform restoration of Dsg3-expression and functional epithelial structure. Thus, SCT with BM-derived HSCs is a promising treatment for inherited epidermolysis bullosa and other severe inherited epidermal diseases in humans.

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Age and gender dependent differences of collagen XVII shedding in human keratinocytes

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Collagen XVII (BP180) represents the major autoantigen in blistering dermatoses ofthe pemphigoid group, like bullous pemphigoid (BP). The immunodominant epitopes of these autoantibodies are mainly located within the juxtamembranousNC16a-domain of collagen XVII. This NC16a domain is also constitutively cleaved by disintegrin-metalloproteinases (ADAMs), resulting in the release of collagen XVII ectodomain from the cell surface. Since this highly stable and soluble ectodomain is also target of collagen XVII autoantibodies, shedding is likely involved in the initiation of autoimmunity in the elderly.

The aim of this study was to answer the question whether there are differences in the expression und shedding of collagen XVII in keratinocytes of young and old healthy persons. Therefore, primary keratinocytes were prepared from skin biopsies of 14 younger (4.4 ± 3.5 years) and 16 older (66.1 ± 10.9 years) male volunteers as well as six older female volunteers (73.1 ± 13.7 years). The total RNA and protein was extracted and expression levels were determined by RT-PCR and Western blot analysis. Our analysis revealed that collagen XVII expression was not different in keratinocytes of young and old donors, but shedding of collagen XVII was significantly increased by 70% in the cells of older persons. Interestingly, we also have detected a gender specific increased shedding rate in female keratinocytes.

To explain the higher shedding rates, the expression of relevant ADAMs and their physiological inhibitors (TIMPs) were analyzed. So far we have seen no significant differences between young and old persons on RNA level, but further analysis on protein level will most likely reveal differences.

In addition, we have also analyzed the expression of plasminogen activators in the keratinocytes, since we have previously shown that serum derived plasmin cleaved collagen XVII within the NC16a domain in injured or blistering skin in an ADAM independent manner. It revealed significantly higher mRNA expression of urokinase-type plasminogen activator in keratinocytes of older people, which points to an indirect role of activated plasmin in the pathogenesis of autoimmune diseases due to increased skin fragility and wounding in the elderly. These results may contribute to the explanation of increasing susceptibility to autoimmune skin disorders in the elderly.

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Metabolic changes in a mouse model of an autoimmune disease

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Within the last decades the incidence of chronic inflammatory diseases increased markedly. Hence, treatment of patients with chronic inflammatory diseases is of growing clinical relevance. In addition, inflammations can often be directly linked to other diseases. An inflammatory response normally targets exogenous pathogens, but in case of autoimmune diseases the immune response is misled to endogenous proteins. Due to the chronic nature of autoimmune diseases the medical treatment is difficult and long.

In epidermolysis bullosa acquisita (EBA) antibodies against a major structural protein in the skin are formed, resulting in chronic blister formation at the skin. EBA serves as a model for autoimmune diseases providing the advantage that both, antibodies and the autoantigene, are known. In addition, a mouse model is available, i.e. the disease can be induced within mice. [1]

During a disease state cell metabolism is changed by altered protein expression and, as a consequence, increased or decreased concentrations of certain metabolites. By using the mouse model of EBA we aim to identify changes in the metabolom caused by the autoimmune disease. The identification of metabolic pathways related to the disease will provide clues to diagnosis as well as to a deeper understanding of the disease itself.

NMR Proton spectra of sera from mice developing EBA ($n = 14$) as well as two control groups (untreated and control immunized mice, 15 mice each) were obtained. The spectra were subjected to principal component analysis for identifying differences between the three groups. Discrimination between the groups can be achieved, however the discrimination is not as clear as expected and no correlation to the severity of the disease was observed.

Future experiments aim to improve discrimination of the two groups and to identify the substances causing the differences. Furthermore, another mouse model of the disease will be investigated, as well as the effect of diets and environmental factors on the metabolism in disease animals.

Reference: [1] Sitaru C, Chiriac MT, Mihai S, et al., Induction of complement-fixing autoantibodies against type VII collagen results in subepidermal blistering in mice. J Immunol 2006: 177(Suppl. 5): 3461–3468.

P307

A novel method for the visualisation of the amount of sunscreen products applied to skin by *in vivo* attenuated total reflection FT-IR spectroscopic imaging

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There is an ongoing discussion about the applied quantity of sunscreen products. For the measurement of sun protection factors (SPFs) the typical amount of product applied to skin is, according to EU and other international standards, 2 mg/cm^2.Previous studies have unfortunately shown that consumers often tend to use much less than the required amount. Other SPF studies, which demonstrated different exponential and linear relationships between the SPF and the applied amount of sunscreen products, reported a decrease in SPF with a decreasing amount of sunscreen product.

Not only for people with photodermatoses, photoallergies, drug-induced photo-sensitisation or immunosuppression, but for everyone, it is essential to have effective protection against skin damages like sunburn, photo ageing and skin cancer.

Fourier transform infrared (FT-IR) spectroscopic imaging with focal plane array detectors has proved a powerful technique for rapid chemical visualisation of a huge number of different samples. It offers the possibility of combining spectral and spatial information. *In vivo* IR imaging is an important new field of application. In this feasibility study, for the first time the application of this technique is described for the *in vivo* study and visualisation of different amounts of sunscreen products (0.5, 1 and2 mg/cm^2) including the typical amount a consumer normally applies on skin. It could be clearly visualised that the amount mostly applied by consumers is only about one fourth to one third of the required amount of 2 mg/cm^2.

With the resulting IR imaging pictures very demonstrative and convincing material is available for the first time. It can be used for patient education in order to show and assure them how important the right amount of applied sunscreen products is.

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Silver-containing crèmes inhibit the growth of *Candida albicans* and *Malassezia spp*.

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Objectives: Patients with atopic dermatitis (AD) are highly susceptible to cutaneous bacterial, fungal and viral infections. Bacterial colonization with *Staphylococcus aureus* is the most common skin infection in AD. Recently, there has been growing evidence that yeasts of the malassezia genus are involved in the pathophysiology of AD. Studies also suggested that *Candida albicans* has a possible pathogenic role. These yeasts are considered to be part of the normal skin flora; however, they might trigger several cutaneous and systemic diseases. Malassezia has been associated with pityriasis versicolor, seborrhoic dermatitis, dandruff, folliculits and atopic dermatitis. Treatment with antifungal reduces the population of yeasts on the skin, and this decrease would help to break the inflammatory cycle. Silver-containing formulations or dressings have found a broad application in the treatment of colonized or infected wounds due to the broad-spectrum antimicrobial activity of silver. It kills microbes on contact trough multiple mechanisms of action such as inhibiting cellular respiration, denaturing nucleic acids, and altering cellular membrane permeability. Hence, we have tested two silver-containing crèmes according to the JIS L 1902 for antifungal activity.

Method: According to the JIS L 1902 norm samples of 400 mg of the silver-containing crèmes (Sebapharma) were applied on polyester for testing. Polyester and polyester plus crème without silver were used as reference material. The samples were incubated with the *Candida albicans, Malassezia furfur* and *Malassezia pachydermatis* up to 24 h at 37°C under aerobic conditions.

Results: The silver-containing crèmes were able to significantly inhibit the growth of all three yeasts. Already a slight effect on microbial growth by crème alone could be observed. The pH of the silver-containing crèmes influenced their efficacy. Silver-containing crème at pH 7.5 was more effective against *Candida albicans* than at pH 5.5, while it showed a lower capacity to reduce *Malassezia pachydermatis* compared to pH 5.5. Both formulations were equally effective against *Malassezia furfur*.

Conclusions: Atopic dermatitis is a chronic inflammatory skin disease whose initiation and clinical activity is modified by exposure to and interaction with micro-organisms. Defects in the skin immune defense mechanisms such as decreased expression of antimicrobial peptides result in exacerbation of disease activity by skin colonizing micro-organisms particularly *S. aureus* and *Malassezia spp*. Treatment-induced microbial growth reduction has been postulated to improve skin lesions. Support of the innate immune system by silver-containing crèmes during daily skin care could provide a novel approach to the treatment of AD.

P309

Comparison of different biomaterials regarding binding capacity for elastase and antioxidative potential *in vitro*

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Introduction: Non-healing wounds contain elevated levels of neutrophil elastase and free radicals. This overproduction perpetuates the inflammatory phase and results in severe tissue damage as well as degradation of growth factors. These destructive processes prevent wound closure and lead to persisting wounds. It has been shown, that the binding of these active mediators contributes to the treatment of chronic wounds. Modern wound dressings include different types of biomaterials such as collagen, cellulose, chitosan or alginate. The aim of this study was to investigate the binding capacity of these wound dressings for elastase and antioxidative effect *in vitro*.

Materials & Methods: Wound dressings were chosen to represent one of the biomaterial types: bovine collagen type I (Suprasorb C, Lohmann & Rauscher), oxidized regenerated cellulose (Tabotamp, Ethicon), bovine collagen + ORC (Promogran, Johnson & Johnson), chitosan (HemCon Bandage, HemCon Inc.) and alginate (Suprasorb A, Lohmann & Rauscher). The wound dressing samples were cut into equal pieces (0.5 cm^2), taken in a final volume of 1 ml of elastase solution (0.1 U/ml), and incubated up to 24 h at 37°C. Supernatants were collected and stored at –20 °C. The residual elastase activity in the supernatants was determined with the EnzChek Elastase Assay Kit (MoBiTec, Germany). Antioxidant potential was measured using the chemiluminescent ABEL® Antioxidant Test Kits containing Pholasin® specific for superoxide and peroxynitrite (Knight Scientific Limited, UK).

Results: All wound dressings tested significantly reduced elastase activity *in vitro*. Furthermore, all wound dressings exhibited a significant antioxidant potential. The samples were equally effective in inhibiting the formation of reactive oxygen and nitrogen species (ROS/RNS).

Conclusions: Today, a wide variety of wound dressings is available. In general, these dressings are classified as inactive (gauze, fleece material), interactive (hydrokolloids, alginate, hydrogel) and active (protease-modulating matrix, growth factors). Only the last are thought to provide active influence on the chronic wound milieu while the others merely grant a moist wound environment, thermal isolation and protection against invading micro-organisms. However, it is difficult to sort the biomaterial-derived wound dressings into these three classes. Only the collagen and the collagen/ORC product would per se fit the active dressing type. They showed a high binding capacity for elastase and effectively inhibited the formation of free radicals. Nonetheless, alginate, chitosan and ORC alone as well exhibited significant binding capacity for elastase and distinct antioxidative potential. Thus, they should have an auxiliary influence on the healing of chronic wounds.

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Influence of polyethylenimine structure on cytotoxic and genotoxic effects in *Salmonella thyphimurium*

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In the last years the poly cation polyethylenimine (PEI) became an important tool for *in vivo* and *in vitro* transfection experiments. In future, the use of PEI as transdermal drug-delivery system for the treatment of skin diseases is imaginable.

The high cationic charge density of PEI is a key parameter for nucleic acid condensation and is also responsible for the cytotoxicity of PEIs. In the present study we investigated the influence of molecular weight and degree of branching on cytotoxic and genotoxic effects of PEIs in gram-negative bacteria *Salmonella typhimurium*.

Three branched PEIs (5, 25 and 750 kDa) and two linear PEIs (2.5 and 25 kDa) were tested. Two *Salmonella typhimurium* strains TA 98 and TA 100 with frame shift and base pair mutations were selected for the experiments. Cytotoxicity of polyethylenimines was determined by laser nephelometry. Genotoxicity of polyethylenimines was investigated by a modified Ames test (OECD 471) using the plate incorporation method.

Only low cytotoxicity was determined for concentrations up to 1 mg/ml PEI. Cytotoxicity of linear PEIs is not influenced by molecular weight in both strains. In contrast, branched PEIs had a lower toxic effect on TA 98 than on TA 100. Further experiments with higher concentrations are ongoing. Using the Ames test, colonies increased in all samples with increasing incubation time. Under chosen conditions no genotoxicity could be detected for the tested PEIs up to 5 mg/ml.

In conclusion, under the chosen conditions low cytotoxic effects and no genotoxic potential of the tested PEIs could be detected in the *Salmonella typhimurium* strains.

(Branched PEIs were a kind gift of BASF SE, Ludwigshafen)

P311

Interventions for mycosis fungoides - Protocol for a systematic review

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Mycosis fungoides (MF) is the most common subtype of cutaneous T-cell lymphoma. In MF a multitude of therapeutic options are described in the literature mainly without thorough research evidence backing these up.

In 2008 the Cochrane Skin Group as a research part of the internationally active Cochrane Collaboration priorized the need for a systematic review about interventions for mycosis fungoides by an international voting. Within the same year on the German National Level the Joint Committee for Quality Assurance of the Professional Organization of the German Dermatologists (BvDD) and the German Dermatological Society DDG independently judged this title via a blinded delphi-process conducted by this research team as one of the most relevant research issues for a systematic review.

As a result of this a protocol for a systematic Cochrane review has been developed that will be used to search, summarise and assess the flood of research articles concerning therapeutic interventions for MF.

We will include randomised controlled trials of adults with histologically proven mycosis fungoides classical 'Alibert-Bazin' type which compared any local or systemic therapy with either another local or systemic therapy or with placebo. We will search the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE, GOOGLE scholar and available databases of ongoing trials. Two review authors will independently screen titles and abstracts of studies identified from the above sources for the eligibility criteria stated previously. We will attempt to obtain data that were not reported directly from the original researchers. In order to assess for possible reporting bias, we will examine a funnel plot for asymmetry. We will explore potential causes of heterogeneity by performing sensitivity and subgroup analysis.

Where possible, we will conduct a meta-analysis of trials and subgroups, or both, using random effect models. We will present data in the form of forest plots.

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Epidemiology of eczema- A representative German cross-sectional study

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Introduction: Eczema is among the most frequent chronic conditions in childhood and adolescence, affecting approximately 20% of infants and 6% to 10% of adolescents. Studies have identified determinants of eczema in childhood and adolescence, but their relative role is still unclear. It is the aim of this study to identify determinants of eczema occurring in childhood and adolescence.

Methods: Data was drawn from the public use files of the German Interview and Examination Survey for Children and Adolescents (KIGGS), a nation wide cross-sectional representative survey conducted between 2003 and 2006, including a total of 17,641 children aged 0 to 17 years. We investigated the association of abroad set of environmental and lifestyle exposures with ever physician-diagnosed eczema by means of multivariable logistic regression modeling.

Results: The weighted prevalence of ever-physician diagnosed eczema was 13.2% (95% confidence interval (CI) 12.5%–13.9%). In multivariable analysis, age (per year increase Odds Ratio (OR) 1.03 (95% CI 1.01–1.04)), socio-economic position (per increase in socio-economic position OR 1.11, 95% CI 1.00–1.24), problems after birth (any vs. none OR 1.19, 95% CI 1.02–1.38) and parental allergies (any vs. none OR 1.97, 95% CI 1.74–2.22) were positively associated with eczema. Migrant status (OR 0.66, 95% CI 0.51–0.84) and regular maternal smoking during pregnancy were inversely related to eczema (OR 0.62, 95% CI 0.42–0.92). Sex, number of older siblings, breastfeeding and other environmental factors (mould on the walls, pets, East/West) were unrelated to eczema.

Conclusions: In line with previous findings, this study suggests that an atopic constitution as mirrored by a family history of allergies is the strongest determinant of eczema, whereas environmental factors which have been implicated in the pathogenesis of eczema are less important determinants. Being a migrant to Germany seems to confer protection with regard to eczema. The positive association of birth problems and eczema is noticeable. The protective association of smoking during pregnancy with eczema needs further investigation and might be due to social desirability bias and/or self-selection.

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Relevance of occupational UV-exposure for basal cell carcinoma risk in out door-workers: a systematic review of the literature

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The most important risk factor for basal cell carcinoma (BCC) is UV-radiation of the skin. The objective of this study was to systematically analyze the association between occupational UV-exposure and BCC. A systematic review of the literature according to the MOOSE checklist was performed. PubMed (until 05/2009) was searched, supplemented by hand searching and consultation of experts in the field. The association between occupational UV-exposure (i.e. outdoor-work) and BCC risk is presented as odds ratios (OR). A total of 17 articles on 16 relevant epidemiological studies (4 cohort studies, 12case-control studies) were identified. In general, study quality was weak. Most studies failed to consider relevant confounders, were prone to misclassification bias of exposure and did not consider the pattern of occupational UV-exposure. 6 studies described a significant association between occupational UV-exposure and BCC in outdoor workers (OR between 1.43 and 4.9). 10 studies did not show a relevant association between occupational UV-exposure and risk of BCC (OR between 0.78 and 1.4). In 4 of the studies even a tendency towards a reduced risk was found (OR between 0.78 and 0.86). Based on the currently available evidence, the association between occupational UV-exposure and BCC remains unclear. Studies performed so far show a low methodological quality. Moreover, the majority of the studies do not discriminate between BCC entities and affected body areas, which might be crucial for the discrimination between occupationally and non-occupationally UV-induced BCC.

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Quality assurance in the management of occupational dermatoses: evaluation of dermatologist's procedure and hierarchical multi-step intervention

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In Germany, the dermatologist has a pivotal function in the care for patients with occupational dermatitis (OD): by notification of a case with suspected OD to the statutory accident insurance (dermatologist's report) a multi-step intervention is started. It's aimed at ensuring that patients with OD receive preventive measures quickly and appropriate to the stage of their condition. Since 01.09.2007, a research project ('EVA_Haut') is supported by the statutory accident insurances in order to evaluate the quality of dermatological intervention as well as administrative procedures for the first time. Therefore, about 10% of the annually notified cases in Germany with suspected OD in 2007 have been randomly selected (n = 1,600). This random quota sample is taken in relation to the amount of OD-notifications from the different high risk professions. The main cohort comprises cases in which dermatological and/or preventive intervention has taken place within the dermatologist's procedure. Dermatologist's reports submitted to the statutory accident insurance are analyzed anonymously by certified occupational dermatologists by means of a standardized questionnaire (double-review following a random allocation matrix). Patients and dermatologists involved are interviewed as well. In cases without intervention after notification, the anonymised medical documents are evaluated in a similar fashion, however in single-review. At the same time, the implementation of the hierarchical multi-step intervention scheme by the insurance-administrations is evaluated over the one year follow-up period in each case. Main criteria are the course of OD, job loss and costs of the procedures. To date, n = 995 of 1,600 notifications led to consecutive preventive intervention within the dermatologist's procedure, n = 552 did not. Acceptance by patients and dermatologists is good: the response rate of questionnaires is 55% (patients) and58% (dermatologists), respectively. Preliminary results show that both groups consider the dermatologist's procedure and the cooperation with the statutory accident insurance as effective. The research project offers the unique opportunity to analyse the quality and interaction of dermatological and administrative procedures in the management of occupational dermatoses in order to further improve interdisciplinary prevention in OD-patients.

P315

Occupational non-artificial UV-light exposure is an independent risk factor for the development of squamous-cell carcinoma- Meta-analysis suggesting a new occupational skin disease.

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Background: Exposure to UV-light is the most important risk factor for squamous-cell carcinoma (SCC) of the skin. In Germany, about two million individuals are exposed to work related non-artificial UV-light and may therefore be at increased risk for the development of SCC. One reason why SCC is not included in the list of occupational diseases in Germany is the lack of a comprehensive qualitative and quantitative review to summarize the epidemiological evidence concerning the relationship between occupational UV exposure and SCC risk.

Methods: Based on a systematic literature search in PubMed (until 05/2009) supplemented by hand search the association between occupational UV exposure (i.e. outdoor work) and the development of SCC was analysed. Standardized assessment of eligibility and data abstraction was done independently by two reviewers. The association between occupational UV exposure and SCC risk is presented as odds ratios (OR) and corresponding 95% confidence intervals (95%CI).Qualitatively homogenious studies were pooled using a random-effects model.

Results: We identified 15 relevant epidemiologic studies (4 cohort studies, 8 case-control studies, 3 cross-sectional studies). 12 studies reported a positive association between occupational UV exposure and risk of SCC with OR>3 in 6studies and OR 1.5 to 2.0 in another 6 studies. 3 studies did not find a relevant association (OR 1.0 to 1.4). Meta-analysis of 11 studies with sufficient data indicated a strong pooled relationship between occupational UV exposure and the development of SCC (OR 2.18; 95% CI1.58 to 3.02).

Conclusions: There is consistent and convincing epidemiological evidence that individuals with chronic occupational UV exposure have a more than 2-fold increased risk to develop SCC of the skin compared to indoor workers. Therefore, SCC should be considered as an occupational disease in persons with long-term outdoor work and preventive measures should be offered to outdoor workers.

P316

Epidemiology of pediatric psoriasis: A representative German cross-sectional study.

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Background: Psoriasis is a common inflammatory skin disease frequently manifesting in childhood, adolescence, or early adulthood. The impact of psoriasis on health related quality of life and the associated economic burden are both remarkable. Despite the significance of psoriasis in current clinical research and in every day clinical practice, representative studies about the prevalence and risk factors of psoriasis in children and adolescents are missing.

Methods: We performed a cross-sectional study utilizing the public use files of the German Interview and Examination Survey for Children and Adolescents (KIGGS); a nationwide representative survey including a total of 17,641 children aged 0 to 17. In addition to the prevalence of psoriasis (diagnosis by physician) in children we investigated the association of a broad set of environmental and lifestyle exposures (i.e. socioeconomic position, maternal health problems in pregnancy, perinatal health status, breastfeeding, and day care, number of siblings) and psoriasis by means of multivariate logistic regression modeling.

Results: Data of 16,500 children with data on lifetime prevalence of psoriasis were analyzed. Lifetime prevalence of psoriasis was 1.37% (226 / 16,500 participants reported having ever been diagnosed as having psoriasis). The final logistic regression model (adjusted multivariate analysis) revealed independent significant associations between psoriasis and perinatal infections (odds ratio [OR] 2.24; 95%-confidence interval [CI] 1.17–4.30) and perinatal jaundice (OR 1.72; 95%-CI1.18–2.52). Children with high socioeconomic position (SEP) were at significantly lower risk for psoriasis compared to children with low SEP (OR 0.59; 95%-CI0.38–0.91). Maternal infections during pregnancy tended to increase the risk of psoriasis (OR 2.34; 95%-CI 0.95–5.81), whereas sex and breastfeeding had no effect.

Conclusions: Psoriasis is a relevant health problem in children and adolescents. Our finding that perinatal infections of the child and maternal infections during pregnancy both seem to increase a child's risk to develop psoriasis is in concordance with the hypothesis that microbiologic and immunologic factors acting very early in life might be critically involved in the pathogenesis of psoriasis.

P317

Psychiatric and cardiovascular co morbidity in psoriasis - a population-based case-control study from Germany

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Background: Psoriasis may significantly reduce quality of life. Previous studies reported an association of psoriasis and cardiovascular risk factors and cardiovascular events. The extent to which psoriasis is associated with psychiatric morbidity and the role of psychiatric co morbidity as a potential confounder of the association between psoriasis and cardiovascular morbidity requires further investigation.

Objectives: To study the association between psoriasis, psychiatric morbidity, and cardiovascular morbidity.

Patients/Methods: We conducted a case-control study utilising an interdisciplinary administrative outpatient database from Germany. Patients with confirmed diagnosis of prevalent psoriasis within the study period (2003–2004) (n = 3,147, mean age 57years) were individually matched by age and sex to 3,147 controls without psoriasis. The relationship of psoriasis with psychiatric morbidities (depression, stress-related disorders, behaviour disorders, schizophrenic disorders), cardiovascular risk factors (diabetes, hypertension, obesity, dyslipidemia), and cardiovascular events (myocardial infarction (MI), stroke) was investigated using logistic regression models.

Results: Crude analyses suggested an association of psoriasis with depression, stress-related disorders, behaviour disorders and cardiovascular risk factors, but not with MI (odds ratio (OR) 1.14; 95%-confidence interval (95%CI) 0.81–1.62) or stroke (OR 0.97; 95%CI 0.61–1.54). Multivariate models controlling for age, sex and consulting behaviour indicated that psoriasis is independently associated with depression (OR 1.49; (95%CI) 1.20–1.86), stress-related disorders (OR 1.41; 95%CI1.22–1.62), behaviour disorders (OR 1.58; 95%CI 1.05–2.39), diabetes (OR 1.2195%CI 1.04–1.40), hypertension, (OR 1.34; 95%CI 1.18–1.51), dyslipidemia (OR 1.29;95%CI 1.07–1.55), and obesity (OR 1.63; 95%CI 1.39–1.90).

Conclusions: Psoriasis appears to be independently associated with major psychiatric disorders and with cardiovascular risk factors, but not with cardiovascular events in this German representative sample.

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Cardiovascular risk factors in children and adolescents with psoriasis.

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Background: Epidemiologic research indicates that psoriasis is associated with hypertension, diabetes and other cardiovascular risk factors in adults. However, it is still unclear whether these associations constitute a causal relationship or not. One reason for this uncertainty is a lack of studies on the association between psoriasis and cardiovascular risk factors at an early stage in the natural history of psoriasis, i.e. in young populations.

Methods: We performed a cross-sectional study based on the public use files of the German Interview and Examination Survey for Children and Adolescents (KIGGS), a nationwide representative survey including a total of 17,641 children aged 0 to 17.We analyzed the likelihood of overweight/obesity, elevated blood LDL-cholesterol(>130 mg dl^{-1}), elevated HbA1c (>6.1), and mean blood pressure in children ever vs. never diagnosed as having psoriasis by a physician by means of logistic and linear regression models adjusted for age and sex.

Results: Data from 16,500 children with data on the occurrence of psoriasis were analyzed. 226 children and adolescents (1.4%) reported having previously been diagnosed with psoriasis. Mean (SD) age of participants with and without psoriasis was 9.9 (5.0) years and 8.4 (5.1) years, respectively ($P < 0.001$). 53.1% of participants with psoriasis and 49.1% of participants without psoriasis were female ($P = 0.24$).Children with psoriasis tended to be more frequently overweight/obese than children without psoriasis (adjusted odds ratio (OR) 1.30; 95%-confidence interval (95%CI) 0.94–1.81; $P = 0.11$). LDL-cholesterol elevation (OR 1.04; 95%CI 0.65–1.65; $P = 0.88$), increased HbA1c (OR 0.87; 95%CI 0.62–1.22; $P = 0.42$), mean systolic blood pressure (psoriasis vs. no psoriasis 110 mm Hg vs. 107 mm Hg; $P = 0.47$), and mean diastolic blood pressure (psoriasis vs. no psoriasis 67 mm Hg vs. 66 mm Hg; $P = 0.90$) were not related to psoriasis.

Conclusions: In this population-based German sample of children and adolescents none of the investigated cardiovascular risk factors was significantly associated with psoriasis. Considering previous findings of an association between psoriasis and cardiovascular risk factors in adults, our study suggests that patients with psoriasis develop an adverse cardiovascular risk factor profile in adulthood - either as a result of chronic psoriatic inflammation or resulting from adverse behavioural/lifestyle factors possibly related to psoriasis. Ideally, longitudinal studies with incident cases of psoriasis and repeated measurement of cardiovascular risk factors are needed to clarify the causal relationship between psoriasis and cardiovascular disease.

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Human Beta-Defensin (hBD)-2 Skin Washings Useful for Non Invasive Epidermal Inflammation Analysis in Patients with Seborrheic Eczema

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Seborrheic eczema is a common inflammatory skin disease with a high rate of relapse. Until today, the severity of the disease is judged using soft clinical scores or invasive skin biopsy analysis. For the first time we established a standardized skin washing method followed by ELISA for characterizing washing fluids derived from lesional skin from patients with seborrheic eczema. As biomarker we chose hBD2antibodies, known to be expressed only in inflamed epidermis (e.g. in psoriasis vulgaris). Skin washing samples were collected from the face of 25 patients with seborrheic eczema before and after a four week treatment with either 2% ketoconazole cream ($n = 12$) or 1% pimecrolimus cream ($n = 13$) twice daily. The patients were monitored weekly for clinical symptoms using facial investigator global assessment score. Both treatment groups showed significant clinical improvement. The hBD-2 content of the skin washings from the lesional sites revealed a significant reduction of the chosen biomarker ($P = 0.0266$). Washing samples taken from the non-lesional skin of the same patient group did not show changes in hBD-2 content ($P = 0.2165$). Our results indicate that treatment of seborrheic dermatitis led to normalization ofhBD2 expression. HBD-2 may be a marker for inflammation and disturbed epidermal differentiation in seborrheic dermatitis. The two different treatment groups revealed a reduction of hBD-2 expression in the ketoconazole group with a P value of 0.2032, and in the pimecrolimus treated group with a P value of 0.0367. The improvement of clinical symptoms became significant one visit earlier in the pimecrolimus treated group than in the ketoconazole group, correlating with the more significant P value. This demonstrates that both hBD-2 analysis of skin washings and clinical scoring were more favourable in the pimecrolimus treatment group. We suggest that skin washings analysed for hBD-2 might be a new, useful, and non-invasive tool for analysing epidermal inflammation and differentiation stages inpatients with seborrheic eczema.

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Differentially expressed microRNAs as potential regulators of human hair follicle cycling

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Understanding and manipulating the molecular 'hair cycle clock' that rhythmically transforms hair follicles from a rapidly growing (anagen) into a regressing (catagen) and then into a relatively quiescent organ (telogen) constitutes one of the key challenges of basic and clinically applied hair research. Since microRNAs regulate skin morphogenesis and hair follicle development in mice, we hypothesized that microRNAs could also serve as up-stream regulators of human follicle cycling. In the current pilot study, we report the first microRNA expression profile of human scalp hair follicles, and compare this profile between anagen VI and spontaneously developing catagen hair follicles under serum-free organ culture conditions. We identify significantly up- or down-regulated microRNA species that are related to known pathways regulating the anagen-catagen transformation (e.g. miR-17, -101,-107, -185, -187, -202, -208, -215, -302a, -381, 510), and specifically search for fluctuations in the level of microRNAs that interact with the mRNA of recognized key hair growth regulators. Our findings suggest that spontaneous fluctuations in the intracellular microRNA level, which rhythmically suppress the translation of key hair cycle-regulatory proteins, are an integral component of the elusive 'hair cycle clock'.If confirmed in subsequent functional studies, targeted manipulation of intra follicular microRNA levels could become a powerful new therapeutic tool in the management of human hair growth disorders. In addition, hair follicle organ culture offers an excellent, clinically highly relevant model for microRNA research in a prototypic human ectodermal-mesodermal interaction system.

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Assessment of pruritus intensity: correlation between visual analogue scale (VAS), numeric rating scale (NRS) and verbal rating scale (VRS) in patients with chronic pruritus

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The most commonly used tool for self-report of itch intensity is the visual analogue scale (VAS). Similar measurement tools are the numeric rating scale (NRS) and verbal rating scale (VRS). We herein present data of the first study in which reliability and concurrent validity of VAS, NRS and VRS in chronic pruritus were investigated.200 randomly selected patients of our out-patient department (88 m, 112 f, mean 62.06 years) recorded their pruritus by VAS (10 cm line), NRS (0–10) and a four-point VRS scale. 25 patients stated no itching on VAS=0 (mean point difference [MPD] to NRS 0.70), 113 patients rated itching as low on VAS (0.1–3.9, mean 1.45; MPD0.52), 25 patients as moderate on VAS (4.0–7.9, mean 5.58; MPD -0.02) and 18patients as severe pruritus on VAS (8.0–10, mean 8.76; MPD -0.26). Pearson's correlation coefficient for VAS with NRS was 0.939; $P < 0.01$. On the VRS, 26 patients stated to have no itch ('0') which was scored on average as 0.11 (VAS) and 0.10(NRS). 96 patients stated to have low ('1') pruritus (mean VAS/mean NRS:1.36/1.90), 54 patients to have moderate ('2') pruritus (mean VAS/mean NRS:4.28/4.83) and 16 patients to have severe ('3') pruritus (mean VAS/mean NRS:8.79/8.56). 9.5% did not record their pruritus intensity by VAS, 2.5% not by NRS and 4.0% not by VRS. Spearman's correlation coefficient for VAS with VRS was 0.788 and for NRS with VRS 0.859; $P < 0.01$. In sum, NRS and VAS showed a very high correlation and concurrent validity with a low point difference of mean 0.39, SD 0.96.Thus, both scales can be used in clinical trials to assess valid data of itch intensity course. The NRS is easier to understand and handle for patients; VAS needs detailed explanations. The correlation of VRS with both VAS and NRS also showed moderate to high concurrent validity; however, the discrimination of itch intensity on the VRS is not as sensitive as on VAS and NRS.

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Epidemiology of occupational skin disease in the Saarland, Germany

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Background: Occupational skin diseases represent the largest fractional share of all occupational diseases in many countries. Establishing surveillance schemes is hence important. The purpose of this study was to collect demographic and clinical data on suspected occupational skin disease in one federal state of Germany (Saarland).

Methods: In the federal state of the Saarland, Germany, cases of suspected occupational skin disease were centrally assessed in the federal office for environmental and occupational protection from 01.07.1999 to 31.12.2008. Demographic data and data on sick leave, exposure and diagnostics were collected in an assessment questionnaire completed by the government occupational physician for each case.

Results: In total, 1262 cases were assessed of which 54% were female. The mean age was37 years (age range 16–71 years). They worked in the following occupations: healthcare services (24%), food industry (18%), metal workers (17%), cleaning services(8%), mining (7%), hairdressers (7%), construction workers (6%), printing/chemistry(5%), others (8%). The majority suffered from irritant contact dermatitis (58%), followed by allergic contact dermatitis (17%), atopic dermatitis (11%) and contact urticaria (3%). Allergic contact dermatitis occurred most frequently in metal workers, health care workers and hairdressers. Irritant contact dermatitis occurred most frequently in health care workers, food industry workers and metal workers. Almost all cases of contact urticaria occurred in health care and food industry workers. 37% of all cases had clinically relevant type-IV-sensitizations to a large number of allergens such as nickel (II)-sulfate or fragrance mix.

Conclusion: This study demonstrates the occupations which are mainly affected by skin disease and the subtypes of dermatitis that mainly occur. The data form a basis upon which targeted prevention measures can be developed.

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Epidermal Wound Healing: An interdisciplinary approach towards understanding cell migration dynamics

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Wound healing is a highly complex process, calling upon multiple cell types to work in unison to re-establish the tissue barrier. The development of model systems to explore the cues leading to alterations in single cell and tissue responses have become the focus of multiple research disciplines. As cell organizational patterns have been found to influence overall cellular responses [1], a closer look at cell patterns during re-epithelialization, the governing cues and the resulting cell responses could provide further insight into healing mechanisms and wide spread cellular interactions. To study such mechanisms with regards to whole tissues and the role of individual cells, sample imaging has emerged as a key method from the ability to maintain the spatio-temporal organization patterns of a sample. Conventional imaging techniques apply invasive, often costly and laborious methods to visualize the spatio-temporal cell organization profiles, while inadvertently also disrupting the native phenotype(s) of a sample. However, spectroscopic imaging techniques have displayed much promise for circumventing these drawbacks through non-invasive, label-free sample imaging, combined with useful chemical insight in the form of multivariate spectra. With this in mind, an in vitro model system for re-epithelialization was spectroscopically imaged at varying time-points, providing insight into the activity of individual cells in the context of simulated tissue layers. Cells were mechanically disturbed through stimulated wounding to induce re-epithelialization, leading to the release of sequested chemical stimuli. Subsequent cell activity was imaged using Fourier-Transform Infrared spectroscopy and micro-Raman imaging to visualize and explore cell communication, polarization, migration and the resulting organizational profiles during re-epithelialization. Here, keratinocyte organizational profiles were found to dramatically differ during re-epithelialization and lead to phenotype changes in migrating cells that persisted post-wound closure. Additionally, changes in lipid expression profiles were found to be tailored to specific functions and polarize in leading cells during migration. Moreover, as spectroscopic imaging techniques provide substantial information with respect to sample distribution, critical knowledge related to non-contact cell communication profiles was obtained through transcriptome analysis. Combined, a comprehensive and quantitative view of re-epithelialization was obtained.

Through the combined use of optical imaging techniques and transcriptome analyses, thorough insight of transcriptome fluctuations and cell phenotypes during the onset, maintenance and inhibition of cell migration were obtained. By better understanding cues involved in cell migration, advancements in tissue engineering, biomarker identification, treatments and diagnostics, particularly with applications in cancer metastases and healing deformations will follow suit.

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A multidisciplinary Training Programme for Patients with Chronic Pruritus:Outline and preliminary results

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Chronic pruritus (> 6 weeks) is a worldwide symptom and a burden in many dermatological, systemic and psychosomatic/psychiatric diseases. Patients with chronic pruritus frequently endure a long and complicated disease course, failure of therapy and a substantial reduction in quality of life. Psychological mechanisms maybe involved in eliciting and coping with chronic pruritus. Treatment of pruritus aims to be etiological, but as a primary illness it is symptomatic. The needs of patients with chronic pruritus are diverse. Multi-disciplinary educational and psychological training programmes aim to improve patients' understanding of the disease, raise motivation to apply more adaptive self-care measures, and consequently improve quality of life. A multi-disciplinary training consisting of dermatological, health-educational and psychological modules and based on the needs of patients with chronic pruritus was developed. The programme comprises four weekly meetings lasting two hours each and has been offered to 44 individuals so far. The programme provides information about the medical fundamentals of the skin, the multi-factorial nature of pruritus, current diagnostic procedures, the epidemiology of pruritus and therapeutic avenues for the relief of pruritus. Patients also learn about and discuss more adaptive behavioral response patterns to pruritus and the interrelationship between stress and pruritus. An established relaxation technique is practiced. During all modules patients are encouraged to share their experiences with other patients. All 44patients evaluated the four session programme. Of these 17 also completed a quality of life (QoL) instrument, measures of psychological functioning and rated the pruritus severity before and after the training. Patients rated the programme as a highly expedient means to increase their understanding of pruritus and felt empowered to better deal with the pruritus sensation in daily life. QoL measured by the ItchyQoL improved somewhat. The hospital anxiety and depression scale (HADS), used as a measure of psychological functioning, was subject to little change. The severity of pruritus as measured by the visual analogue scale appeared better after the training. Maintenance of health through educational programmes, such as the one presented here, can be considered an important complementary measure in the field of medicine and psychosomatics, which should also be applied to patients with chronic pruritus. Preliminary results suggest improvements in QoL and reported pruritus severity.

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Estimating the prevalence of chronic itch: How common is the symptom?

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Not only is pruritus the most common symptom in dermatology it is also frequently found in many systemic, neurological and psychosomatic/psychiatric conditions. Epidemiological data on chronic pruritus, that is itch lasting for more than six weeks, is sparse but important in order to understand burden and risk factors of this distressing symptom. Given this sparsity of data, the epidemiology of chronic pruritus research group (ECPRG) based at the University Hospital Heidelberg, Germany, set out to undertake a population based study on chronic pruritus. Here, we report on the prevalence of chronic pruritus. Addresses of 4503 German citizens were obtained from the registers of local residents in two cities and 6 rural communities in Southwest Germany at random. A previously validated questionnaire was sent out by mail. A reminder was sent after 2months to all non-responders. The remaining non-responders were contacted by telephone if their number was listed in the telephone directory; if the number could not be obtained a third reminder including a shortened version of the questionnaire was sent. Of the 4503 individuals contacted 105 were ineligible to participate leaving a total sample of 4398. The response rate successively increased with each reminder. The total response rate was 58%. The point-prevalence of chronic pruritus was 13.5% (95% CI 12.2% - 14.9%), the 12-months prevalence 16.4% (95% CI 15.0% - 17.9%) and the lifetime prevalence 22.0% (95% CI 20.4% – 23.7%). Compared to other large scale studies measuring prurigo or itch in general the estimates arrived at in this study appear higher. However, a recent employee based study reported an even higher point-prevalence. This is the first study investigating the prevalence of chronic itch in the general population. The results from this study suggest that burden of chronic pruritus in the general population is substantially higher than previously believed. It appears as if more than a fifth of the population has suffered from chronic pruritus at least once in their life.

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Can models of health behaviour inform us on the mechanisms underlying skin protection behaviour in patients with suspected OSD?

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Occupational skin diseases are a significant public health concern. Little is known about the cognitive representations individuals with occupational skin disease have towards measures of personal skin protection and occupational safety and whether they change during an intervention. Social-cognitive models of health behavior have been widely used to predict and explain health behaviour. No study so far has applied these models to skin protection behaviour in individuals with a suspected OSD. We aimed to evaluate whether social cognitions as embodied by the theory of planned behaviour (TPB), the prototype-willingness model (PWM) and the health-action-process-approach (HAPA) become more favourable during a three-week tertiary in-patient individual prevention programme (TIP) and whether the models predictions hold in a setting to which the models have not been applied. We used a longitudinal design and assessed the respective model's variables at admission (T0) to and discharge (T1) from a TIP three weeks later. Once patients had been back to work for a continuous period of 4 weeks (T2) implementation of skin protection measures was measured. The questionnaires were developed by elicitation interviews and tested in a pre-test. 150 patients were recruited and 117were followed up until T2. The intervention provides a variety of health-educational and psychological units. Drop-out analyses revealed no significant differences between those who were and those who were not followed with diagnosis being the exception. Drop-outs were more likely to have a diagnosis of atopic dermatitis. The questionnaire appeared reliable. Most socio-cognitive variables significantly improved during the intervention. Multiple regression analyses showed that the models assumptions regarding the prediction of intention to perform skin protection behaviour were largely confirmed. Path analyses tested whether T0 and T2 variables predicted adherence to skin protection behaviour once patients had been back to work for 4 weeks. Prediction of skin protection behaviour using the TPB, PWM and HAPA compared to previous studies investigating other health behaviours. This is the first study attempting to explain skin protection behaviour in patients with OSD from a socio-cognitive perspective. Strong evidence was produced for the utility of the tested models. The results also emphasize the importance of health-educational and psychological interventions because they can help patients form a strong motivational basis and foster self-regulatory skills underlying regular skin protection behaviour.

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5D-intravital tomography-a non-invasive tool for *in vivo* analysis of human skin

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Atopic Dermatitis (AD) is an inflammatory disease of human skin. Its pathogenesis is still unknown; however, dysfunctions of the epidermal barrier and the immune response are regarded as key factors for the development of AD. Impaired skin barrier allows penetration of pathogens, and an increased exposure of allergens or hazardous substances provoking an enduring inflammatory response. Previously the invasive technique of taking biopsies with all its risks and inconveniences as a surgical procedure has been one of the most important methods to confirm and objectify clinical findings.

In our study we applied a novel intra vital multi photon tomography (5D-IVT), equipped with a spectral-FLIM module for the five dimensional *in vivo* and *ex vivo* analysis of human skin affected by AD. The technical setup was established within the framework of BMBF project 5D-IVT by JenLab GmbH. We investigated different skin areas of 40 pro-bands in five visits over three month to get a survey of diverse states of inflammation and to compare various medical treatments. We detected the characteristic skin morphologies of AD such as spongiosis or acanthosis. Moreover, the degree of these morphological alterations correlates with the disease activity measured by SCORAD. In addition to the morphologic skin analysis FLIM technology gain access to the metabolic status of the epidermal cells referring to the NADH specific fluorescence lifetime. FLIM analysis revealed a shift of the mean fluorescence lifetime (τm) of NADH, indicating an increased metabolic activity in AD affected skin. Within an *ex vivo* approach we have investigated cryo-sections of human skin with or without barrier defects. Spectral-FLIM allows the detection of auto-fluorescent signals that reflect the pathophysiological condition of the defect skin barrier. In our study we measured an auto-fluorescence with an excitation maximum at 710 nm and an emission maximum at 460 nm. The τm value was shown to be different between healthy and affected skin. Application of the 5D-IVT allows non-invasive *in vivo* imaging of human skin with a penetration depth of 150 μm. We could show that affected skin could be distinguished from healthy skin by morphological criteria, by FLIM and by spectral-FLIM. Further studies will evaluate the numerous applications of the 5D-IVTtechnology as a diagnostic tool and to monitor the therapeutic efficacy.

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Locating mesenchymal stem cells in the human hair follicle dermal papilla by focal plane array-fourier transform infrared spectroscopy

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Mesenchymal stem cells (MSC) in the human hair follicle have been shown to be multi-potent and take part in cutaneous wound healing by providing new fibroblasts. They can be found in the dermal papilla (DP) and the connective tissue sheath (CTS) which surrounds the hair follicle. Since they are easily accessible and are abundant, their harvesting is highly desired in regenerative medicine. Their isolation and characterization is however hindered by the lack of known specific antigens for identifying them, apart from the expression of nestin protein which is an intermediate filament also found in neuronal stem cells. Focal plane array (FPA) – fourier transform infrared (FTIR) spectroscopy provides a label-free and non-destructive method for obtaining macromolecular information from biological samples. As molecular bonds absorb infrared at specific wavelengths, and the level of absorbance depends on the quantity of the specific bonding, measurements of infrared absorbance provides qualitative and quantitative results of bio-molecules present in the sample. In order to identify and to characterize the MSC, we applied FPA-FTIR to map scan over the centre of the hair bulb, over the DP and the CTS below the DP. The scanned area is sub-divided into 8192 small sub-sections, with each section approximating the size of a DP cell (5–10 μm) *in situ*. An FTIR spectrum is recorded in each sub-section, forming a hyper-spectral data cube. This hyper-spectral data cube was analyzed using the chemometric technique unsupervised hierarchical clustering analysis. Clustering individual spectra based on their chemical similarities. From this, bio-molecular information in each region could be extrapolated and de-convoluted to identify pheno-typical traits. Based on the current knowledge on mesenchymal stem cells from the literature and from our nestin immunostaining results, we postulate that the MSC in the DP should be centered in the DP, contain high level of fatty acids (lipid reserve for when proliferation is required) and high level of proteins (signaling molecules and transcription factors are required to maintain stem cell state) and may be identified by the presence of phosphates (PO2-) related to DNA methylation state. By performing hierarchical clustering analyses on the spectra using different criteria, producing spectral clusters, and finding the region of high fatty acids and proteins, we successfully singled out an area which is likely to be the MSC-containing region within the DP. Individual spectra can be analyzed further for stem cell related features. We aim to verify this method as a valid way to narrowing down an MSC-containing region by staining the FTIR map scanned sample using anti-nestin antibody.

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Comparison of the serum tumor markers S100 and melanoma inhibitory activity (MIA) in the monitoring of melanoma patients undergoing dendritic cell vaccination

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Tumor markers constitute helpful tools in the monitoring of cancer patients. They can be employed in the follow-up of disease-free patients to detect recurrent disease, as well as to survey the response to therapy in advanced patients. We retrospectively compared two serum markers for malignant melanoma, S100 and melanoma inhibitory activity (MIA) which we had used to monitor melanoma patients undergoing dendritic cell vaccination (103 patients, 29 stage III and 74 stage IV).Serum was obtained at each visit and staging was performed at usual intervals. The sensitivity and specificity of S100 and MIA to detect progressive disease was analyzed with receiver operating characteristics (ROC) curves. When a relapse was discovered by imaging in the stage III patients receiving adjuvant DC vaccination both markers often remained below the threshold. In contrast, in patients undergoing therapeutic DC vaccination an increase of S100, MIA, or both markers was a strong indicator of disease progression. Likewise, a remission under therapy was suggested by decreasing tumor markers. In the receiver operating characteristics, the sensitivity to detect progression and remission of MIA was superior to the sensitivity of S100. In some patients with considerable tumor burden, one or both of the markers remained below the threshold. We conclude that S100 and MIA are highly sensitive tumor markers for malignant melanoma which appear useful to detect progression of metastatic disease but less so relapse in disease-free patients. In terms of sensitivity, MIA was superior to S100in our analysis. Considering that in some patients with metastatic disease only one of the markers was elevated above the threshold, we recommend parallel measurements of S100 and MIA in the monitoring of melanoma patients receiving systemic therapy.

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Nanoscaled analysis of native human stratum corneum affected by atopic dermatitis applying atomic force microscopy

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Recently, we could show that atomic force microscopy (AFM) is an appropriate nanotechnological tool for 3-dimensional imaging of native human stratum corneum (SC) at a nanometric scale. In a previous study we compared the SC of young and healthy skin with aged and atrophic skin. Analyzed skin samples were obtained from individual volunteers by tape stripping and were imaged by AFM without further preparation. In our present study we investigate skin atrophy induced by long-lasting treatment with topical corticosteroids in the context of atopic dermatitis therapy. We further examined potential skin recovery upon pimecrolimus treatment instead of the application of corticosteroids. In complementation to AFM analysis severity of skin atrophy was correlated to the dermatophot score. Long-lasting treatment with topical corticosteroids leads to a decrease of the corneocyte volume in comparison to non-corticosteroids treated skin. In atrophic skin, corneocytes have an average height of 0.78 ± 0.082 μm ($n = 15$), a volume of 536 ± 11.9 fl ($n = 15$) and a surface area of 736 ± 223.3 μm^2 ($n = 15$). In healthy skin the average height of corneocytes was 0.94 ± 0.175 μm ($n = 15$) the corneocyte volume was 1132 ± 451.0 fl ($n = 15$) and the surface area was 1314 ± 485.5 μm^2 ($n = 15$). Moreover, we could show that the surface of corneocytes derived from atrophic skin were decorated with cellular protrusions which were completely absent in healthy skin sections. The surface of corneocytes from healthy volunteers was characterized by a dense network of filamentous structures. Medical treatment of atrophic skin regions with topically applied pimecrolimus over a period of about 12 months leads to a partial recovery of the skin reflected by an improvement of the dermatophot score. Skin recovery was also measurable by AFM imaging as indicated by an overall morphology change of single corneocytes back to a cell volume and an average height comparable to healthy skin. Moreover, the above mentioned cellular protrusions were almost absent and filamentous structures were detected. In conclusion, we could show that AFM is an innovative tool enabling high resolution 3D-imaging of native human stratum corneum. Our data show significant changes of single corneocytes morphological upon topical corticosteroids treatment which is partial reversible upon pimecrolimus therapy

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Participants of online surveys on health care in dermatology have something incommon: the critical view

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Aim: To compare assessments on health care of patients with skin diseases gathered online versus data obtained by paper-and-pencil surveys.

Methods: In four distinct web surveys a total of 4428 patients (1946 with psoriasis, 542 with acne, 1348 with rosacea and 592 with atopic dermatitis) filled in the online version of a questionnaire on clinical features, therapy, disease burden and patient benefits. Subsequently, difference analyses were conducted between these diseases. Furthermore, the web-based outcomes were compared to paper-and-pencil based data on 5251 patients with psoriasis, atopic dermatitis and rosacea obtained with the same outcomes measures.

Results: Across all patients who participated online, more women took part (55.8% to74.3%). Topical treatment was used by 94.7% to 99% of patients. Most experienced with systemic therapeutics were patients with acne (64.6%), followed by atopic dermatitis (47.0%), rosacea (38.7%) and psoriasis (29.1%). There were only minor differences regarding satisfaction with treatment (1 = very satisfied to 4 = very dissatisfied) between atopic dermatitis and rosacea (each2.8 ±0.9), psoriasis (2.9 ±0.9) and acne (3.1 ±0.9). Only small differences between the diseases were also found regarding patient defined treatment benefits and perceived treatment success. The lowest patient benefit (range 0–4) was found in acne patients (1.1 ±1.0), followed by atopic dermatitis (1.2 ±1.0), rosacea (1.3 ±1.1) and psoriasis (1.4 ±1.2). The patient benefit across the diseases was only marginally above the cut-off-value of 1 (minimum clinically relevant benefit). Both, patient benefit and treatment satisfaction were considerably lower in the web based surveys compared to the paper-and-pencil surveys.

Discussion: The results indicate that patients with acne evaluate their health care situation worse than patients with other skin diseases. However, these differences are small indicating that the specific disease is of minor importance. Striking results are the marked differences between web-based and paper-pencil-based data with regard to the patients' evaluation of their health care situation and their treatment benefits. This implies that the evaluation of health care depends on the way of surveying. Further studies are needed in order to clarify whether the differences are 'real' or just induced by the evaluation methods.

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Quality indicators and health care index: New methodology for the assessment of guideline-compliant care in chronic wounds.

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Background: Chronic wounds are of great socio-economic importance. However, evidence for the efficacy of modern wound treatments is scarce. Moreover, there are very few data on the quality of health care in wounds. One of the major reasons for this is the lack of valid methods to evaluate the quality of wound care.

Aim: 1) Development of quality indicators and a single index of quality for wound care,
2) Application of the indicators as measures of guideline-compliant care.

Methods: Criteria for the quality of care were derived from the German national (AWMF) guideline and the international guidelines. A prioritized list of criteria was developed by means of a nationwide Delphi consensus among wound experts (physicians and nurses), including 20 indicators which define 'optimal treatment'. These were transformed to a score from 0–100. The study included a consecutive, representative sample of $n = 520$ patients with chronic leg ulcers of any origin in the metropolitan area of Hamburg. Patients were approached in wound clinics, office-based practices, nursing homes, home care services and special ambulances for homeless persons and drug users, thus providing a large spectrum of 220 healthcare providers in total. All patients were interviewed, photographed and personally examined by trained wound experts. The patients were asked to fill a questionnaire addressing quality of life (QoL), experiences with prior therapy and quality of care. Predictors of quality of care were analyzed by multivariate regression analysis.

Results: A total of 502 out of 520 patient records could by analyzed, including 63%venous, 23% mixed, 2% vasculitic and 12% other leg ulcers. Taken together, a high proportion of patients (78.6%) were treated with modern wound dressings. Also pain and compression therapy mostly accorded to guidelines. However, there were deficits in diagnostics (e.g. angiology, biopsies, pain measurement) and in the concomitant wound treatments. A high proportion of patients still suffered from marked reductions of QoL. The average health care index was below 60, and only about 61% of patients reached at least 60 index points. Major predictors of high quality of care were age (younger patients) and treatment by a wound expert. By contrast, wound characteristics, health insurance and social status were not predictive.

Conclusions: In spite of mostly 'lege artis' topical treatment, many patients in the area of Hamburg are not treated satisfyingly according to guidelines. The quality indicators developed by the Delphi panel and the 'health care index' proved to be helpful for quality evaluation, reflecting implementation of evidence-based guidelines. These parameters enable a precise planning of measures for improving health care in chronic wounds.

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Introduction of community skin cancer screening in Germany: First data on theimpact for dermatologists.

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Background: On July 1st 2008, community skin cancer screening (cSCS) was established in routine care covered by the German statutory health insurances (SHI).

Objective: Gain first data on the impact of cSCS on health care provision in German dermatology practices.

Methods: Standardized questionnaires were sent to about 2,000 German dermatology practices. Questions included the kind and extent of SCS, the impact on drug prescriptions and surgery, and personal attitudes on SCS.

Results: In total, $n = 693$ (34.7%) questionnaires were returned. On average, every praxis performed $n = 354$ SCS per quarter, the mean payment being 21.50 EUR. About 78% of practices named an increase in number of SCS since introduction of cSCS (by 36.7% on average). About 54% of practices performed SCS under SHI payment, connected with individual health services ('IGeL'), 38% only SHI services and 8% exclusively 'IGeL'. In 85% of practices, the number of surgical procedures increased since the start of cSCS (on average by 23%). 40% of practices had an increase in drug prescriptions related to SCS (on average by 7%). 32% of dermatologists were satisfied or very satisfied with cSCS, 40% rather or completely unsatisfied. 29% of dermatologists would prefer cSCS covered exclusively by SHI, 29% only as 'IGeL' and 42% in a combined fashion. A majority of dermatologists (70%) regarded the quality of health care of patients with skin cancer in Germany since the introduction of cSCS better.

Conclusion: Dermatologists in Germany have mainly accepted their role as major Providers of community skin cancer screening. However, the framework and basic conditions need further improvement.

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Two-dimensional pO2 mapping of split-thickness skin graft donor sites

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Aims: Many experimental and clinical observations have shown wound healing to be impaired under hypoxia. However, the role of oxygen in wound healing is not yet completely understood. Therefore, oxygen-dependent quenching of luminescence using transparent planar sensor foils was used for two-dimensional measurements ofpO2 in acute wounds over the time course of physiological wound healing.

Methods: The split-skin graft donor site was used as a standardized model for acute (syn. physiological) wound healing. In twelve patients the surface pO2 was measured at 1, 6, and 14 days after split-skin harvesting using two-dimensional luminescence lifetime imaging (2D-LLI) of palladium (II)-meso-tetraphenyl-tetrabenzoporphyrin bound to polystyrene-co-acrylonitrile (Pd-TPTBP-PSAN) immobilized in aD4-hydrogel matrix on transparent polyvinyliden chloride (PVdC) foils.

Results: Split-thickness donor site pO2 on day 1 amounted to 57.90 ± 5.49 mmHg, to 22.14 ± 6.18 mmHg on day 6 and to 6.32 ± 3.24 mmHg on day 14 after harvesting ($n = 12$). pO2 decreased significantly from day 1 to day 6 postoperatively ($P<0.001$).There was also a highly significant decrease of pO2 from day 6 to day 14 after the operation ($P<0.001$). Regional differences in oxygen tension could be visualized within split-thickness donor site wounds and are shown using pseudo color images.

Conclusions: Knowledge on pO2 values during wound healing of split-thickness donor sites is important basic knowledge on the time-course of oxygen tension during acute wound healing. The presented method of 2D-LLI enables clinicians to map regional differences in pO2 and shows the future potential of LLI in studies of heterogenous chronic wounds.

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Genomics analysis of expression of stratum corneum lipid metabolism pathways associated with aging and responses to cosmetic ingredients

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Aims: To evaluate effects of human skin intrinsic and photo-aging on expression of gene pathways involved in stratum corneum (SC) barrier lipid metabolism, and whether cosmetic ingredients, can affect SC lipid pathways *in vitro*.

Methods: Full thickness biopsies were taken from sun-protected (buttock, intrinsic aging) or sun-exposed skin (outer forearm, combined photo- and intrinsic aging) from ten young (18–20 years old) and ten aged (60–67 years old) female subjects; older subjects had moderate to severe forearm photodamage. Skin equivalent cultures formed using dermal fibroblasts and epidermal keratinocytes from neonatal skin or 54 year old females were treated topically up to 48h with aqueous solutions of skincare active ingredients. Total RNA from each natural or *in vitro* skin sample was purified, labeled and hybridized to affymetrix gene chips. Following statistical analysis, bioinformatics focused on expression of genes and pathways specific to metabolism of SC lipids and their extracellular release via lamellar bodies.

Results: In both intrinsically and photo-aged skin, there was a down-regulation in expression of genes involved in SC lipid biosynthesis and metabolism. As compared to young skin, epidermal cholesterol synthetic and influx pathway genes were significantly down-regulated, while the major efflux pathway, ABCA1, was up-regulated. Similarly, the expression of genes involved fatty acid synthesis and uptake, sphingolipid biosynthesis and processing, and lamellar body secretion were significantly down-regulated. In *in vitro* skin models, the skin care actives produced expression of SC lipid pathways in directions opposite to the effects of intrinsic and photo-aging, suggesting improvement in SC barrier function.

Conclusions: The coordinated down-regulation of SC lipid pathways at the level of gene expression is consistent with previously reported global decreases in SC lipids in aging skin, and likely contributes to the decreased capacity of aged skin to maintain and repair the epidermal barrier. The actives induced coordinate up-regulation in expression of SC lipid genes, suggesting increased levels of these key lipids available for SC maintenance and repair.

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Cyclosporin A inhibits nucleotide excision repair via down-regulation of thexeroderma pigmentosum group A and G proteins which is mediated by calcineurin

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Cyclosporin A (CsA) inhibits nucleotide excision repair (NER) in human cells, a process that contributes to the skin cancer proneness in organ transplant patients. We investigated the molecular mechanisms of CsA-induced NER reduction by measuring the xeroderma pigmentosum (XP) mRNA and protein expression of all known XP genes (XPA-XPG). Western blot analyses revealed that XPA and XPG protein expression were reduced in the cytosol of GM00637 fibroblasts exposed to 0.1 μM or 0.5 μM CsA, respectively. Nuclear XPA and XPG protein expression was completely inhibited. Other XP proteins were not down regulated by CsA. The immunosuppressive effect of CsA is modulated via calcineurin. Using RNAi we found that calcineurin knockdown in GM00637 fibroblasts similarly down-regulated XPA and XPG protein, suggesting the involvement of calcineurin-dependent signaling in XPA and XPG protein regulation. CsA-induced reduction of NER in GM00637 fibroblasts could be complemented by over expression of either XPA or XPG protein assessed by host cell reactivation (HCR) and transfection of XPA or XPG cDNA-containing plasmids. Likewise, XPA-deficient fibroblasts stably corrected with XPA (XP2OS-pCAH19WS) did not retain the inhibitory effect of CsA on NER. In contrast, CsA reduced NER in XPC-deficient fibroblasts (XP4PA-SV-EB) complemented with XPC. Further, CsA treatment of GM00637 fibroblasts reduced XPG but not XPA mRNA expression. Our data indicate that the CsA-induced inhibition of NER is a result of the down-regulation of XPA and XPG protein in a calcineurin-dependent manner. The fact that CsA affects XPA protein but not XPA mRNA expression suggests the involvement of at least another protein regulation pathway besides transcriptional regulation.

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Distinguishing and characterizing hair follicle tissue layers using fourier transform infrared spectroscopy

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Fourier Transform Infrared Spectroscopy (FTIR) can provide information on the macromolecular make-up of biological samples, based on the absorption of infrared light at specific wavelengths depending on the chemical bonds that are present in a bio-molecule. The specific absorptions constitute spectra that can be considered as molecule-specific as fingerprints. These can be used to identify one cell/tissue type from another, for examples cancer and non-cancer cells. By sub-dividing an area of a biological sample into smaller sub-sections, and collecting infrared spectra from each sub-section, an image based on the bio-molecular information of the cells can be constituted. Using an FTIR instrument coupled to a microscope, we successfully collected infrared spectra from a human hair bulb from a human scalp skin cryosection. After applying chemometric analyses using Cytospec™ software, based solely on the spectroscopic hence bio-molecular variations between sub-sections, we were able to discern the tissue layers in the hair bulb. Each spectral cluster can be identified as a different tissue layer, and an average spectrum from each cluster was calculated. Each average spectrum provides information on the levels of protein, lipid, fatty acid, carbohydrate and nucleic acid contents, as well as secondary structure of proteins, so that the level of presence of keratin, collagen, proteoglycan etc. can be extracted and compared. FTIR microspectroscopy imaging is to our knowledge applied to discerning hair follicle layers for the first time, and based on this success, we aim to apply this label-free and non-destructive technique to other aspects of hair follicle and skin research, so that single cell variations within a tissue layer can be investigated, and effects of gene knock-out's and application of diffusible factors/drugs uncovered.

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Risk awareness does not correlate with behaviour: A pilot study on the prevalence of sunbed use and user characteristics*

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Objectives: Skin cancer is caused by ultraviolet radiation. Indoor tanning facilities are totally avoidable cancer risks. Although sunbed use is common in many developed countries, little is known about the prevalence of indoor tanning and the characteristics of sunbed users. Therefore, this study aims at estimating prevalence of use of indoor tanning facilities to identify risk groups and motives in a population-based survey.

Methods: The SUN-Study (Sunbed-Use: Needs for Action-Study) is a pilot study conducted in a German city (Mannheim) and contains data on indoor tanning practices. Five hundred adults aged 18 to 45 were randomly selected and asked about their tanning practices, their motivation and risk perception, and the compliance of staff with international sunbed use recommendations.

Results: 47% of the 18–45 year olds stated having visited an indoor tanning facility atleast once in their lives. Every fifth was a current user. The average current user reported a mean of 15 visits per year (median =10). The reports ranged from 1 to 120 times per year. Respondents exposed themselves to the indoor UVR for 13.6 +/– 4.3 min each time they used a sunbed. Therefore, the total average UV exposure per current user was around 3.4 h per year (range 0.13 h–36.0 h per year). Current sunbed users were predominantly female, employed, had completed vocational school (or an equivalent certification), were smokers, participated primarily in individual sports and had skin types III or IV. Prevalence of use was not reduced in risk groups for skin cancer: The known risk groups, namely individuals with a history of sunburn, pigmentation marks and familial melanoma risk, do not use solariums significantly less often. Risk awareness of users equaled that of non-users. The poor quality of services and advice provided by many solariums was alarming.

Conclusions: Our results highlight specific potential risk factors for intervention that should be examined in other settings among different populations. It can be concluded that appropriate measures to change tanning habits need to be identified. Legal regulations could be one option. As a first achievement, the results of this pilot study resulted in an utilisation ban for minors in Germany from 2010 on.

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Analysis of data from skin-cancer screening - epidemiological study on 90,000 employees in Germany

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Background: Epidemiological data are essential in planning medical care. For skin cancer, only a few studies have been published on this topic in Germany so far, most of them not population-based. The objective of the study was to obtain reliable data on the frequency of skin diseases in an extensive working-population.

Methods: From 2001–2008, skin cancer screenings including whole body examinations were performed by dermatological consultants in more than 300 German companies from various branches. Point prevalence was calculated by a retrospective analysis of the screening-data. All diagnoses are based on the clinical examinations exclusively, thus representing 'tentative diagnoses'.

Results: The data of 90,880 employees (46.7% female) ages 16 to 70 years (a mean43.5 years) were available for analysis. Suspicion of malignant skin changes arose in $n = 2{,}889$ screening participants. Prevalence was: malignant melanoma 0.20%; basal cell carcinoma 0.86, squamous cell carcinoma and various forms of precancerous 2.08%. For all diagnoses prevalence was rising with increasing age. This was especially noted for squamous cell carcinoma, which had a prevalence of 0.14% up to the age of 40 years and of 3.45% in the age-group of 60 years and older.

Discussion and Conclusion: Lesions suspicious for skin cancer were detected in 3.18% of all employees indicating that skin cancer screening in this population is worth while procedure. Selection bias regarding the participating companies and employees as well as the lack of histologic diagnostics in the findings must be discussed as a potential limitation. In general, this study provides a population-related assessment of skin cancer prevalences in Germany. The results correspond largely to smaller studies published and may be of assistance in healthcare planning and allocation-decisions.

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Working with itch: epidemiology of pruritus in a cohort of 11,700 employees in Germany

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Background: Chronic pruritus is a frequent and challenging symptom in dermatology. It can severely affect the quality of life and poses a considerable socio-economic problem. Treatment of chronic pruritus is difficult since in many cases the underlying cause cannot be treated or simply is unknown. In spite of being a relevant medical problem, only very few data on the prevalence of pruritus in the general population have been published. Moreover, there are no population-based data available regarding the quality and severity of itch in the persons affected.

Aims: To assess the prevalence, quality and severity of chronic itch in the German working population.

Methods: A cross-sectional observational study was conducted from 1/2008 to12/2008 on employees, aged 16–70 years, of 144 German companies who were screened in-house for skin cancer. Whole-body clinical examinations and interrogations were conducted by trained dermatologists. Moreover, standardized questions characterizing pruritus were applied.

Results: 11,732 persons examined (53.2% male) were suitable for analyses. Point-prevalence of pruritus (at least six weeks prior to data collection) was 16.8%.Gender differences were small (male 16.1%; female 17.5%) but prevalence rises with increasing age from 12.3% (16–30 yrs.) to 20.3% (61–80 yrs.) A quarter of the affected persons suffered from pruritus for more than 5 years. The single most frequent localizations of pruritus were arms and legs; the whole body was affected in 16%. On a scale from 0 to 10 mean intensity of itch was rated 5.8. 47% had never attended a doctor for itch; 94% had never applied any treatment. Study participants who suffered from pruritus frequently or constantly also perceived a significantly higher intensity of itch. Chronic pruritus was more persistent in persons suffering from dermatological co morbidities, e.g. atopic eczema or psoriasis.

Conclusions: A potential selection bias cannot be excluded completely. However, the age distribution of this cohort corresponds largely to the data of the general working population in Germany so that the findings can be considered representative for persons at working age. Chronic itch thus is a prevalent symptom in the general population. There is a high proportion of persons who suffer from itch - even for along time and to a large extent - who have never attended a physician and who are not under medical treatment. For this group a considerable extent of medical undersupply can be hypothesized.

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Comparative analysis of perifollicular mast cells in alopecia areata and normal human scalp skin

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Alopecia areata (AA) is a hair follicle (HF)-restricted, T-cell-dependent autoimmune disease, characterized by hair loss. The typical histological feature of AA is an inflammatory infiltrate around the anagen hair bulb, composed of lymphocytes, mast cells (MCs) and other immune system cells. The collapse of constitutive immunoprivilege in the proximal HF, which results in premature anagen termination and catagen involution, plays a major role in AA pathogenesis. Since an increase in the number of MCs was described in AA patients, it has been hypothesized that they take part in AA pathogenesis. To clarify the role of MCs in the development of AA, we stained paraffin sections of AA patients and healthy human scalp skin for mast cells using c-kit immunohistochemistry, Tryptase and Ki-67 double-staining and Toluidine blue histochemistry. This was followed by quantitative statistical analysis of the MCs number, degranulation and proliferation in designated reference areas of the HF connective tissue sheath (CTS) and in the parafollicular dermis. In addition, we studied the distribution of MC infiltrates in the upper dermis, dermis and hypodermis. Our results demonstrate an increase in the number of MCs and MC degranulation in AA patients compared to control skin, most pronounced in the CTS and in the early stage of the disease. MC proliferation is directly proportional to MC numbers. Additionally, we show an increase in the number of immature MCs in AA patients, confirming that MCs are able to proliferate in the skin. These findings strengthen the role of MCs in the development of AA, either by participating in an inflammatory response, inducing a rapid catagen induction, or by directly initiating the HF immune privilege collapse. Therapeutic modulation of MCs within the human HF might, therefore, become a new strategy in the management of AA.

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Local immune imbalances in hidradenitis suppurativa skin

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Hidradenitis suppurativa (HS, acne inversa) is a chronic inflammatory follicular skin disease of the apocrine sweat glands-bearing areas that originates from the distal hair follicle (HF) epithelium. We hypothesized that the excessive release of pro-inflammatory signals from the distal outer root sheath (ORS) plays an important role in HS initiation, subsequent follicular occlusion and the perpetuation of unchecked, chronic inflammation. Since bacterial super infection is a common feature of HS, we wished to clarify whether components of the HF's innate immune system, that can transmit potent pro-inflammatory signals, show any abnormalities in HS. The expression of selected antimicrobial peptides (AMPs) (psoriasin, lysozyme, cathelicidin and human beta defensin 3 (hBD3)), MHC class I and II, macrophage migration inhibitory factor (MIF), alpha-melanocyte stimulating hormone (α-MSH),tumor necrosis factor-alpha (TNF-α) or mast cells and CD8+, CD4+ cells was examined by immunohistochemistry or toluidine blue histochemistry. Scalp skin sections from 18 HS patients were compared to 10 normal specimens as controls. Immunohistomorphometry and statistical analyses revealed significant increase in immunoreactivity (IR) for psoriasin, cathelicidin, hBD3, α-MSH, MIF and TNF-α in the epidermis and psoriasin in the HF ORS of HS skin compared to controls. No significant changes were observed for MHC I. The staining intensity of TNF-α and cathelicidin was also significantly increased in the dermis. Lysozyme and hBD3immunoreactivity was down-regulated in the epidermis, whereas TNF-α showed a decrease in the dermal parts of the ORS. These phenomena were most pronounced in active inflammatory, as opposed to scarring, HS lesions. The total number of MHC class II+. CD4+, CD8+ and mast cells, as well as the percentage of degranulating mast cells was also up-regulated in HS-affected skin, in particular in highly inflammatory HS lesions compared to HS lesions in late stages of scarring. The observed enhancement of AMPs, α-MSH, MIF and TNF-α expression in the distal HF and the increased number of CD4+, CD8+ cells and the number of perifollicular mast cells with activation status in HS lesions invite the hypothesis that AMP, α-MSH, MIF and TNF-α overexpression and CD4+, CD8+ or mast cell stimulation are involved in HS pathogenesis and that both are promising future targets for HS therapy.

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Cost-of-Illness and Cost-Effectiveness in Patient Care of Venous Leg Ulcers in Germany

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Objectives: Apply two different methodical approaches investigating the cost-of-illness of venous leg ulcers treated in Germany.

Methods: In 31 specialized dermatological, surgical and general-medical wound centers a nationwide cross-sectional study was conducted. Data on resource consumption and associated direct costs of the patients with confirmed diagnosis of venous leg ulcer(s) were collected by the physician or patients. In Greater Hamburg, a second cross-sectional study was performed in 147 institutions (hospitals, forester homes and nursing services, practices, homeless shelters and drug units). In this study a detailed anamnesis with wound status and treatment was documented inpatients with all types of ulcus cruris genesis. There from additional resources consumption could be calculated, and associated costs could be determined. Direct, indirect and intangible costs were the main criteria. In both studies, patients were asked about their health related quality of life.

Results: In study 1, $n = 218$ patients participated and had a mean age of 69.8 years (study 2: $n = 502$, 71 years). The wounds on average had existed for 7 (9) years. EUR9, 569 (EUR 10,624) per year and patient was the mean total cost amounted for the ulcer disease. They consisted of EUR 8,658 (EUR 9,851) direct and EUR 911 (EUR772) indirect costs. EUR 7,631 (EUR 9,122) of the direct costs was accounted for by the statutory health insurance (SHI) and EUR 1,027 (EUR 730) by the patients. Inpatient costs, necessary non-drug treatment as well as physicians and nurse fees were the major cost factors for the SHI. Health-related quality of life was severely impaired, implying intangible costs.

Conclusion: Regardless of different recruitment strategies and cost calculations in both studies comparable direct, indirect and intangible costs were specified. Differences can be traced to a large extent to sample characteristics of the study patients. Relevant disease costs were produced by the chronic leg ulcers and thus underline a necessity for an early and qualified disease management in all areas of health care.

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Epidemiology, co morbidity and economics of psoriasis in Germany: Analysis of health insurance data from 1.3 Mio. persons including 34,000 psoriatics.

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Aim: Assess the prevalence, co morbidity and economic factors of psoriasis based on routine data of a statutory health insurance company in Germany.

Methods: Routine data were collected from the Gmünder Ersatzkasse (GEK) which is represented all over Germany. One-year-prevalence of psoriasis was assessed for a closed cohort of continuously insured persons in 2005. Analysis was repeated for the year 2007. Co morbidities were detected by a-priori specified ICD-10 diagnoses. Persons without diagnosis of psoriasis served as a control.

Results: 1,344,071 continuously insured persons were identified for the year 2005.The One-year prevalence of psoriasis was 2.53% (men 2.71, women 2.31). After adjustment for age by using the German resident-population of 2006 as the standard, the standardized prevalence rate was 2.72 (men 2.79, women 2.57). Up to the age of 80 years the prevalence was rising with increasing age, being highest for the age-groups from 50 to 79 years (range 3.99%–4.18%). Insured persons up to 20years had a prevalence of 0.73%. There was an almost linear increase of psoriasis prevalence from the newborn age to 18 years. Regional differences showed up after stratification by broad categories (1 digit) of zip codes: Highest prevalence-rates were seen in the north-western (2.78%) and lowest in the southern region (2.17%) of Germany. There were markedly increased rates of co morbiditiy. Overall, 19,663 persons (57.9%) with psoriasis and $n = 451.755$ (34.5%) without psoriasis (non-Pso) showed atleast one co morbidity related to metabolic syndrome or chronic inflammatory disease. The most frequent co morbidity was hypertension (35.6% in Pso vs. 20.6% in non-Pso), followed by hyperlipidemia (29.9% vs. 17.1%). Increased prevalences were also found e.g. for the diagnoses 'metabolic syndrome' (2.9-fold), 'Crohn's disease' (2.1-fold) and 'diabetes mellitus' (2.0-fold). These rates were already significantly higher in children. The data from 2007 showed comparable results. Patients with psoriasis showed increased costs both due to skin disease and due to co morbidity. Major cost drivers were inpatient treatments and drug treatment.

Conclusions: Psoriasis is frequent, of socio-economic importance and associated with significant co morbidity related to the metabolic syndrome. This implies an elevated risk of severe complications potentially reducing life expectancy. Thus, there is a need for early and accurate detection of co morbidity in patients with psoriasis. Dermatologists should be aware of their role as gatekeepers to further interdisciplinary diagnostics and treatment. Psoriasis networks (PsoNet) and continuous education is important tools for better health care in these patients.

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