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Abstracts

P001
Diagnosing latex sensitizations with recombinant latex allergens – the Immunosolid-phase allergen chip (ISAC®)

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Latex allergies can be diagnosed by using different test methods such as IgE quantification, Western Blot (WB), CAST (cellular antigen stimulation test), and invivo methods (skin prick test (SPT), glove use test) 1. A sophisticated method is the measurement of specific IgE against single allergens found in natural latex. These allergens are commercially available as recombinant allergens. Phadia AB (Uppsala, Sweden) provides two methods for detection of specific IgE against recombinant latex allergens: the conventional ImmunoCAP® (Phadia, Freiburg, Germany) test system (available recombin antallergens: Hev b 1, Hev b 3, Hev b 5, Hev b 6.01, Hev b 6.01, Hev b 6.02, Hev b 8, Hev b 9, and Hev b 11) and the Immuno Solid-phase Allergen Chip (ISAC®, Genosence Diagnostics. Freiburg, Germany), which enables measurement of specific IgE against 103 different allergens from diverse sources including five latex allergens (Hev b 1, 3, 5, 6 und 8).

The aim of this study was to compare the diagnostic sensitivity and specificity of the ISAC® test kit and the conventional Hev b 5 spiked ImmunoCAP® latex extract. Only 22 of 40 subjects with known hand eczema and positive latex SPT, WB and CAST showed sensitizations against at least one latex allergen on the ISAC® (sensitivity ISAC® 55%, sensitivity ImmunoCAP® latex extract 70%). The mainly detected sensitization was against Hev b 6 (n = 12). When the serum samples were tested with all recombinant ImmunoCAP® allergens, three additional sensitizations against latex could be detected compared to the ISAC®. One of these additional latex sensitizations was due to Hev b 9 which is not provided on the ISAC® allergy chip.

Additionally, the ISAC was used to evaluate fruit sensitizations against peach, kiwi, celery, apple, and carrot in latex sensitized subjects. These fruits are known to cross react with latex allergens. Nine of the 22 subjects with latex sensitization detected by ISAC® were also sensitized to fruits (mostly Bet v 1 homologues in peach and apple). All of them further showed sensitizations to birch allergens (Bet v1 and Bet v2), the ImmunoCAP® sx 1 (atopy marker) and the profilin Hev b 8, indicating that these patients primarily suffer from a pollen-fruit-syndrome.

P002 (V22)
Establishment of an IgE- and mast cell- (histamine and platelet-activating factor) dependent humanized mouse model for allergic gut inflammation

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Development of humanized mouse models is of great interest to study allergic diseases and their treatments especially immunological interventions that may involve species-specific aspects. In this study we developed a humanized mouse model of allergen-specific gut inflammation and analyzed the underlying immunological mechanisms. NOD-scid γc-/- mice were injected intraperitonally with human PBMC from grass or birch pollen allergic donors together with the respective allergen. After 3 weeks sera were collected for detection of human total and allergen-specific IgE. Then, mice were challenged with the allergen rectally and gut inflammation was monitored histologically and by a high resolution videomini-endoscopic system. No difference in human cell distribution and human total IgE in mice sera could be observed between PBMC-treated and PBMC plus allergen-treated mice. However, allergen-specific IgE as well as allergen-specific proliferation and cytokine production after restimulation *in vitro* could only be demonstrated in PBMC plus allergen-treated mice. Compared to control mice receiving no human cells, human PBMC-recipient mice showed a significantly higher allergen-associated colitis according to a score evaluating translucent structure, granularity, fibrin, vascularity, and stool, and a stronger histological inflammation of the colon. Again, the highest endoscopic score was observed in mice which had received PBMC plus allergen. Inflammation of the colon was dependent on IgE, mast cells and the mast cell-derived mediators histamine and platelet-activating factor. Our data indicate that allergen-dependent gut inflammation can be induced in humanized mice allowing the investigation of pathopysiological mechanisms of allergic gut diseases and evaluation of therapeutic interventions.

P003
Glutaraldehyde modified allergoids induce diminished T cell responses

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Allergen-specific immunotherapy (SIT) is a clinically effective therapy for IgE mediated allergic diseases. In an attempt to reduce the risk of IgE-mediated side effects, chemically modified allergoids have been introduced to reduce IgE-binding and retain or increase T cell activation.

The aim of the study was to analyze the difference between intact allergens and allergoids, concerning allergen uptake, T cell and basophil activation.

Therefore we incubated human monocyte-derived immature dendritic cells (DC) with Phleum pratense or Betula verrucosa pollen extracts or with the corresponding allergoids, modified with glutaraldehyde or formaldehyde. After cytokine induced maturation the antigen pulsed mature dendritic cells were co cultured with autologous CD4+ T cells. Allergenicity was tested by basophil activation assay (leukotriene release). In addition the uptake of intact allergens and allergoids by immature DC was analysed.

The proliferation and IL-4, IL-10, IL-13 and IFN-gamma production of glutaraldehyde allergoid-stimulated CD4+ T cells were reduced compared to intact allergen- and formaldehyde allergoid- stimulated CD4+ T cells. In line with this, glutaraldehyde modified allergoids were internalized more slowly. Allergoids modified with glutaraldehyde also showed a decreased leukotriene release.

These findings suggest that B cell epitopes of modified allergoids were destroyed most efficiently by modification with glutaraldehyde. Glutaraldehyde modified allergoids however also displayed lower T cell stimulatory capacity, at least in part due to diminished uptake by antigen presenting DC. In contrast, formaldehyde modified allergoids, investigated in this study, seemed to retain both B cell and T cell epitopes and these immunological differences among allergoids may result from differences in modification and aggregation.

P004
Epicutaneously- and orally-induced tolerance to allergens protects from aTh1-mediated colitis

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Crohn's disease, one of the most chronic inflammatory bowel diseases, is characterized by an inflammation which affect the entire gastrointestinal tract and each intestinal layer. The mouse model of TNBS-induced colitis is pathophysiologically similar to Crohn's disease and hence, is suitable for the investigation of cellular mechanisms. In TNBS-induced colitis, mice develop a persistent colitis which is mediated by CD4+ Th1/Th17-T cells. Previously, we demonstrated that epicutaneous and oral applications of subimmunogenic quantities of allergens (e.g. TNBC) results in low zone tolerance (LZT) which prevents the development of a contact hypersensitivity reaction (CHS), a CD8+ Tc1-mediated skin inflammation. In our study, we addressed the questions whether low amounts of allergens may affect immune responses in the entire organism regardless of their route of application and may influence T cell-dependent immune responses in the skin and the gut. Therefore, we analyzed the effect of oral and epicutaneous treatments with low doses of allergens on the outcome of the TNBS-colitis mimicking Crohn's disease in humans. Notably, it was found that the application of repeated oral and epicutaneous subimmunogenic doses of a hapten (TNCB) before sensitisation affected the course of the TNBS-induced colitis and reduced the inflammation in the gut. These results were determined by a significantly decreased score of inflammatory parameters in the intestine evaluated by mini-endoscopy (*in vivo*) and histology of the intestine (e.g. infiltration of inflammatory cells, vessel density, thickness of the colon wall, loss of goblet cells). In addition, a diminished hapten-specific T cell-proliferation and reduced Th1-cytokine production (IFN-g, IL-2) was observed after both, epicutaneous and oral tolerization, indicating an inhibition of the Th1-mediated colitis by LZT. In summary, this study demonstrated that independent of the site of tolerance induction LZT modulates a CD8+ Tc1-mediated skin inflammation (CHS) as well as a CD4+ Th1/Th17-mediated colitis.

P005
Interplay between CD4+CD25+ regulatory T cells, tolerogenic CD11c+ dendritic cells and CD8+ suppressor T cells is critical for tolerance to contact allergens

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Low zone tolerance (LZT) to contact allergens might be a natural mechanism for the regulation and circumvention of allergies in humans. LZT is induced by epicutaneous applications of subimmunogenic doses of haptens. Previous research in the mouse model has identified CD4+ T cell-related IL-10 to be required for the generation of CD8+ suppressor T cells which prevent the development of contact hypersensitivity (CHS). In this study, we analyzed the role and function of naturally occurring CD4+CD25+FOXP3+ regulatory T cells (nTregs) in LZT. We demonstrated that depletion of nTregs during tolerization (by use of anti-CD25-Ab, DEREG mice, cyclophosphamide) completely abolished the development of LZT resulting in apronounced CHS response (significant ear swelling, hapten-specific T cell proliferation, Tc1 cytokine pattern). Transfer experiments revealed that CD4+CD25+ Tregs are activated by hapten exposure but exhibited an allergen non-specific function. In addition, experiments using the CD11c-DTR mouse-model for depletion of CD11+ DC and adoptive transfers of CD11c+ DC revealed that lymph node-related tolerogenic CD11c+ DC are critical for LZT. Notably, absence of activated Tregs during the induction of LZT resulted in loss of the tolerogenic phenotype of CD11+ DC, indicating a pivotal interaction between nTregs and CD11c+ DC which is critical for the development of the tolerogenic properties of DC. Adoptive transfer experiments of CD8+ T cells generated in the absence of nTregs or of tolerogenic CD11c+ DC showed that the consecutive activation of both, nTregs and tolerogenic CD11c+ DC was followed by the generation of CD8+ suppressor T cell preventing the development of the allergic skin inflammation (CHS). Analyses of the regulatory mechanisms of nTregs demonstrated that Treg-derived IL-10 is critical for induction tolerogenic CD11c+ DC as transfer of IL-10+CD4+CD25+ nTregs derived from WTmice, but not of IL-10- nTregs allowed for the induction of tolerance in T cell-deficientmice. Our data demonstrate that an interaction between activated nTregs and CD11c+ DC mediated by IL-10 is critical for tolerance to contact allergens and for the modulation of the network of CD8+ T cell-related immune responses.

P006
A sensitive and specific method to detect IgE to a new mammalian cross-reactive carbohydrate determinant associated with meat and drug allergy

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Background: IgE-reactivity to galactose-[alpha]-1,3-galactose (alpha-GAL) has been shown to be responsible for allergy to red meat, sometimes associated with cat allergy (i.e. pork-cat syndrome) and for anaphylactic reactions to Cetuximab, achimeric mouse-human IgG1 monoclonal antibody approved for cancer treatment. Red meat, cat IgA as well as Cetuximab contain the alpha-GAL epitope.

Objective: To establish a sensitive detection system specific for anti-alpha-GAL-IgE and to investigate sera from several subgroups of patients for the presence of anti-alpha-GAL-IgE-antibodies as prerequisite to determine their clinical relevance.

Patients and Methods: Western blotting of Cetuximab (Merck Serono, Darmstadt, Germany) and cat serum (Bethyl Laboratories, Biomed Laboratories, Hamburg, Germany) under reducing conditions was performed. The galactose-binding lectins RCA and PHA-L and an alpha-GAL-specific monoclonal mouse IgM-antibody were used to identify the alpha-GAL epitope. For inhibition experiments, one strongly reactive patient's serum was pre-incubated with Cetuximab before it was applied to cat serum-separated blotting stripes, which resulted in a dramatic reduction of the reactivity. Twenty five patients with a convincing history of allergic reactions 30 min to 12 h after ingestion of red meat and/or innards with and without clinically relevant cat sensitization, six patients with a probable meatallergy, eight patients with severe urticaria, recurrent and/or delayed anaphylaxis with partially unknown causes were investigated for sIgE to alpha-GAL. A patient with proven Cetuximab allergy served as positive control.

Results: 22/25 sera of patients with symptoms of allergy after ingestion of red meat and/or innards, 5/6 patients with a probable meat allergy, and 6/8 patients with urticaria and anaphylactic reactions with unknown causes were positive in the immunoblot with Cetuximab extract showing IgE-binding of different intensity to adominant immunoreactive band at ca. 50 kDa, which was identified as alpha-GAL by lectins and an anti-alpha-GAL monoclonal antibody.
Conclusion: Whereas the clinical significance of IgE specific for carbohydrate moieties of the MMXF- and MUXF-type (i.e. in insect venoms) is still controversial, anti-alpha-GAL-IgE has been shown to be capable of eliciting serious, sometimes extremely delayed anaphylactic reactions. Our results with sera from redmeat/innards-allergic patients confirm previous evidence that they may be associated with delayed anaphylaxis. Even in sera from patients with severe urticaria and/ordelayed anaphylaxis with partially unknown cause anti-alpha-GAL-IgE can be detected. Their clinical significance as well as the sensitization route is a focus of research. Consequently, a certain glycosylation of therapeutic monoclonal antibodies can harbour the risk for severe hypersensitivity reactions and has to be taken into consideration before treatment initiation.

P007
Penetration of topically applied pollen allergens into Langerhans cells inbarrier disrupted human skin and a prevention method

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Background: It is well known that topically applied pollen allergens (PA) can deteriorate skin condition in sensitized atopic patients. Only recently, in this context hair follicles have been discussed to play an important role. They are surrounded by a dense network of Langerhans cells (LC). Investigations have shown that the penetration of pollen allergens into the hair follicles was significantly reduced when the skin had been treated with specific medical skin care products. But up to now there is no direct evidence that the PA can cross the skin barrier into the living cells.
Objectives: In the present study, the uptake of topically applied PA into LC was investigated on excised human skin after barrier disruption in order to imitate atopic skin condition. PA uptake was compared in skin specimens with and without pretreatment with a special skin care product. As a control the uptake of PA in LC applied on intact skin was investigated.
Methods: Grass pollen allergens were labelled with a fluorescent dye and applied onto excised human skin where the skin barrier was damaged by cyanoacrylate surface stripping. Part of the samples was pretreated with Eucerin pH5 Lotion F. Epidermal cell suspensions were generated and magnetic separation of epidermalCD1c+ cells was performed. The uptake of PA into LCs was measured using laser scanning microscopy. PA were applied on undamaged skin as control.
Results: In contrast to the undamaged skin, pollen allergens could be detected in over 80% of the LC after application on barrier disrupted skin. The pretreatment with the skin care product reduced the penetration of PA to about 10%.
Conclusion: It could be demonstrated that PA penetrate into the viable skin and reach the LC when the skin barrier is disrupted. The use of a specific skin barrier enhancing formulation reduced the penetration of the PA into the LC significantly. For holistic treatment of the type I allergy, greater attention should be focused on the skin as a pathway for PA. The use of a specific skin barrier enhancing formulation could provide an additional allergy prevention strategy.

P008
A novel method for flow cytometry-based analysis of T cell cross reactivity of birch pollen allergen Bet v 1 and homologous food allergens

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Birch-pollen allergy is one of the most prevalent allergic diseases in northern Europe. Among birch-pollen allergic patients approximately 70% experience secondary food allergy e.g. to hazelnuts, carrots, apples or others. Increasing evidence has shown that this oral allergy syndrome (OAS) is mediated by IgE and T cell cross reactivity to proteins of the pathogen related protein family 10 (PR-10) which are homologous to Bet v 1.0101 (e.g. Cor a 1.0401, Dau c 1.0104). In our former studies, the T cell response to recombinant Bet v 1.0101 and Cor a 1.0401 in primary and secondary stimulation induced by human mature dendritic cells was analyzed. Notably, we found a T cell cross reactivity in response to both allergens using the parameter of T cell proliferation in 3H-Thymidine assays. However, this method entails the disadvantage that this readout of T cell proliferation does not allow for discrimination between cells proliferating after secondary stimulation only and those proliferating after primary and secondary stimulation (cross reactivity) on single cell level. To overcome this drawback, we established a new flow cytometry-based method for the analysis of T cell cross reactivity in birch-pollen allergic patients with secondary food allergy to hazelnuts. CD4+ T cells (TC) were first labelled with CellTrace™ Violet (Invitrogen, Dermstadt, Germany) and then primarily stimulated with mature autologous Bet v 1-loaded or unloaded dendritic cells (DC) as control. On day 8, Bet v 1-specific and control TC were recovered and additionally labelled with CFDA (Invitrogen). Subsequently, TC were restimulated with unloaded control DC and allergen-loaded DC (Bet v 1 and Cora 1), respectively. After primary stimulation of CD4+ TC, we detected a higher percentage of proliferating TC in the Bet v 1-specific population as compared to unspecific-activated control TC via CellTrace™ Violet labelling resembling our results of 3H-Thymidine assays. Notably, restimulation experiments revealed a higher proliferation in Bet v 1-specific T cells when restimulated with both, identical (Bet v 1) or cross allergen (Cor a 1) as compared to control TC by CFDA/Violet double labelling. These data indicate that allergen-specific cross reactive T cells were identified by the subsequent use of the fluorescent dyes CellTrace™ Violet and CFDA for assessment of T cell proliferation. Thus, we established a novel flow cytometry-based method for the analyses of T cell cross reactivity on single cell level.

P009
T cell based *in vitro* assays – an important tool for the identification of contact sensitizers

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The replacement of animal testing for the identification of skin and respiratory sensitizers by alternative *in vitro* methods is urgently needed both for ethical and legislatory reasons. One hallmark of sensitizers is the generation of an inflammatory response that leads to the induction of pathogenic T cells. Our goal is the development of *in vitro* T cell based assays within the EU-project Sens-it-iv to identify potential contact sensitizers, assess the potency of sensitizers and to improve the discrimination between sensitizers and irritants.
For this purpose, autologous sorted naive human T cells are primed with autologous immature dendritic cells that are directly modified with the test chemical. As contact sensitizers but not irritants are able to bind to proteins, we use this ability to generate T cell epitopes by modification of the self-protein human serum albumin with sensitizers for subsequent priming of naive T cells Specificity of T cell responses is confirmed by re-stimulation with specific antigens. T cell proliferation and cytokine production are used as readouts to determine allergen specific T cell responses.
In vitro priming of naive human T cells with DCs and contact allergens results in antigen-specific CD4+ and CD8+ T cell responses that allow *in vitro* identification of chemical and protein allergens as determined both by intracellular cytokine staining and analysis of T cell proliferation. Especially the conjugation of allergens to a model protein improved the priming of naive T cells as determined in the proliferation assay.
We show that the improved T cell based *in vitro* assay in combination with protein conjugation is of potential use to complement single cell assays for the identification of contact sensitizers as a second line test within a tiered strategy for the identification of skin and respiratory sensitizers, risk assessment and replacement of animal testing.

P010 (V19)
CD8$^+$ T cells regulate IgE-production *in vitro* via direct killing of B cells using their perforin-granule system

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The perforin-granule system of cytotoxic T lymphocytes (CTL) was previously demonstrated to be involved in IgE control. The mechanisms remain unclear. One possibility is direct antigen-specific killing of IgE-producing B cells by CTL using their perforin granule-system.
To test this, MHC class I molecules of B cells isolated from C57BL/6 mice were passively loaded with an Ovalbumin-peptide (SIINFEKL). Cells were stimulated with LPS and IL-4. CD8$^+$ T cells were isolated from OT-1 mice crossed to perforin knockout (KO) or wildtype (WT) mice and were added to B cells. On day 6 of co-culture, supernatant IgE-levels were determined by ELISA. B cell numbers and viability (FACS analysis) and CTL motility and target contact behaviour (live cell videomicroscopy; Olympus BX61 (Hamburg, Germany), Cell R Image Acquisition System) were determined.
IgE-production was suppressed completely by addition of perforin WT CTL to B cells. In contrast, perforin KO CTL suppressed IgE-production only down to 30% of control levels. KO CTL retained a comparable to WT motility and frequency of target contact formation, yet, they did not influence number and viability of B cells to the same degree as WT CTL which showed a drastic effect.
Our data demonstrate that (i) IgE-producing B cells are killed in an antigen-specific manner by CD8$^+$ CTL without presence of CD4$^+$ T cells or antigen presenting cells in the *in vitro* system chosen, and (ii) this killing effect is significantly reduced in perforin KO-CTL. Thus, perforin may represent one major player in cytotoxic IgE control by directly affecting B cells.

P011
Psychic co-morbidity in adult patients with mastocytosis

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In mastocytosis, several medical conditions like risk of anaphylaxis, chronic pruritus, diarrhoea, osteoporosis and others can alter physical but also mental health. In this study anxiety, quality of life, and general burden was examined in comparison with patients with anaphylaxis due to insect venom allergy. For each group, 54 patients, matched by sex and age were enrolled. Psychic symptoms were recorded by questionnaires STAI, PHQ-D, FKB-20, and SF-36. Patients with mastocytosis show higher psychological burden compared with patients with insect venom allergy. Mastocytosis patients demonstrate significantly higher anxiety levels in the STATE ($P = 0.009$) and TRAIT scale ($P = 0.003$) of the STAI questionnaire and their perception of stress was also significantly elevated as detected by the PHQ stress scale ($P = 0.011$). Quality of life is significantly reduced in mastocytosis patients as detected by lower values for vitality ($P = 0.039$) in SF-36 and vital body dynamic ($P = 0.035$) in FKB-20. Mastocytosis patients seem to be exposed to increased distress and have a lower quality of life. Health care of mastocytosis patients should focus more detailed on these aspects of the disease.

P012 (V26)
Pollen metabolome analysis reveals adenosine as a major regulator of dendritic cell-primed T helper cell responses

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Background: Water-soluble components from pollen modulate dendritic cell (DC) functions such as IL12 secretion, cyclic AMP signaling and migration, possibly contributing to the establishment of a Th2-dominated immune response against pollen. As these effects could not solely be attributed to the previously identified pollen-associated lipid mediators, the pollen metabolome was analyzed for candidate immuno-modulatory substances.
Methods: Fractions of aqueous pollen extracts (APE) were generated by ultrafiltration and were subjected simultaneously to biological tests and metabolome analysis (ultra-high resolution mass spectrometry) and ultra performance liquid chromatography. A low molecular weight fraction of APE (APE < 3 kDa) was used to study effects of pollen-associated adenosine on monocyte-derived DCs cyclic AMP signaling, cytokine response and capacity to differentiate T helper cells.
Results: The <3 kDa fraction of APE comprised thousands of substances, including adenosine in micromolar concentrations. Pollen-derived adenosine mediatedA2-receptor-dependent induction of cAMP and inhibition of IL12 in DCs. APE digested with adenosine-deaminidase failed to mediate IL12 inhibition. DCs of non-atopic donors exposed to APE showed an adenosine-dependent reduced capacity to differentiate Th1 cells and an enhanced capacity to induce Treg and IL10.Tregs primed by APE-exposed DCs were functional as determined by suppression of autologous responder T cells. DCs of atopic donors were less efficient in differentiating Tregs but instead induced IL5 and IL13 in allogeneic T cell stimulation assays.
Conclusion: Out of thousands of metabolites, adenosine is identified as potent immuno-regulatory substance in pollen. It acts on the level of the DC which induces Treg differentiation in nave CD4+ T cells. Notably, this tansmission of tolerogenic pollen-derived signals from DC to T cell seems to be impaired in DCs of atopic patients during pollen season.

P013
Crucial role for human Toll-like receptor 4 in the development of contact allergy to nickel

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Allergies to nickel (Ni2+) are the most frequent cause of contact hypersensitivity (CHS) in industrialized countries. Both a T lymphocyte-specific signal and a proinflammatory signal are required for efficient CHS development. Yet, the molecular mechanisms underlying generation of this co-stimulatory signal are unknown. Moreover, it is unclear why -in contrast to humans- mice require co-administration of adjuvants to generate CHS to Ni2+. Here we demonstrate that Ni2+ induces an inflammatory response via direct activation of human Toll-like receptor 4 (hTLR4), resulting in NF-B-dependent gene expression. Remarkably, nickel-induced TLR4 activation is species-specific since mouse TLR4 (mTLR4) is incapable to generate this response. Studies with mutant TLR4 proteins revealed a requirement of the non-conserved histidines 456 and 458 of hTLR4 for activation by Ni2+ but not by the natural ligand LPS. Accordingly, transgenic hTLR4 expression inmTLR4-/- mice allowed efficient sensitization to Ni2+ and elicitation of CHS.

Altogether, we reveal hTLR4 as crucial receptor for Ni2+ and provide a novel mouse model of contact allergy to Ni2+. Our data describe for the first time the molecular basis of co-stimulation for the most common contact allergen and implicate site-specific hTLR4 inhibition as potential strategy for therapeutic intervention without affecting vital immune responses.

P014 (V36)
MN8001, a dendritic polyglycerol, diminishes allergic type I and type IV reactions in mice

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More than 20% of the world population suffer from allergic diseases such as allergic rhinitis, urticaria, occupational dermatological problems or food induced allergies. Standard allergy treatments often fail to achieve symptom control, necessitating the development of novel drugs. Dendritic polyglycerols have been described to have anti-inflammatory properties. Here, we investigated the effect of the dendritic polyglycerol MN8001 on type I and type IV allergic responses using passive systemic anaphylaxis (PSA) and contact hypersensitivity (CHS) in mice as a model. To first test whether MN8001 affects type I allergic reactions, mice were sensitized by intraperitoneal injection (i.p.) of IgE anti-DNP. After 24 h, the animals were subcutaneously (s.c.) injected with MN8001 (30 mg/kg body weight) or vehicle and PSA was elicited 10 min later by i.p. injection of DNP. Twenty minutes after induction of PSA, vehicle-treated mice had a mean temperature drop of 3.5–0.2°C while that of MN8001-treated mice was only 1.6–0.3°C ($P < 0.005$). Allergic type I reactions are caused by mast cell (MC) activation resulting in the release of biologically potent mediators such as histamine, cytokines and proteases. To test whether MN8001 can inhibit MC degranulation in PSA, we measured mouse MC protease-1 (mMCP-1), which is known to directly correlate with MC activation status. Notably, mMCP-1concentrations in serum of MN8001-treated mice were reduced by ~50% when compared to vehicle-treated animals (6.01 ± 1.1 pg/ml vs 11.8 ± 2.2 pg/ml, $P < 0.05$). We hypothesize that the marked reduction in MC degranulation is mediated by the rapid cellular uptake of MN8001 in MC, as incubation of bone marrow-derived cultured MC (BMCMC) with Cy3-labeled MN8001 showed that $21.96\% \pm 0.067\%$ of BMCMs were MN8001-Cy3+ in FACS analyses after only 30 min of incubation. Next, we investigated the ability of MN8001 to modulate the extent of the MC independent CHS reaction. Therefore, C57BL/6 mice were sensitized to the contact allergen 2,4,6-trinitro-1-chlorobenzene (TNCB) by topical application on the abdomen and after five days challenged by topical application of TNCB on the right ear. Animals having received daily s.c. injections of MN8001 throughout the experiment showed markedly reduced ear swelling by 40% ($P < 0.02$) when compared to vehicle treated mice. However, hapten-specific T cell proliferation was not impaired, and a single dose of MN8001 prior to sensitization did not affect ear swelling responses, both indicating that MN8001 has no significant impact on sensitization. In contrast, multiple-dose injections after the sensitization but not a single-dose prior to the challenge resulted in significantly reduced ear swelling ($P < 0.5$), pointing towards effects in the effector phase. Taken together, the dendritic polyglycerol MN8001 is able to potently reduce allergic hypersensitivity reactions in mice. Additional investigations are needed to further identify the exact mechanisms of action and to better characterize the treatment potential of the substance for allergies in humans.

P015
Allergen-specific blocking activity of IgG antibodies induced by specific immunotherapy

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Allergen-specific immunotherapy (SIT) is a well accepted and highly effective therapy for treatment of immediate type allergies characterized by differentiated immune reaction patterns. Besides the correction of an imbalance between allergen-specific Thelper (Th) cell subsets and allergen-induced IL-10-producing type 1 regulatory T (Tr1) cells, the induction of allergen-specific IgG antibodies (Ab), especially of the IgG4 isotype, is considered to be a critical event in promoting allergen tolerance by SIT. Recently, we showed that SIT-treated birch pollen allergic subjects experiencing a marked clinical benefit still showed an increase of allergen-specific Th2 cells during birch pollen season of the first year of SIT. However, this was balanced by substantially enhanced numbers of allergen-specific Tr1 cells. Of note, no increase of allergen-specific T cell subsets was noticed in the subsequent years of treatment and up to 18 months after withdrawal of SIT, despite a sustained clinical effect.

To better understand the role of humoral changes induced by SIT, we followed the serum concentrations of allergen-specific IgE, IgG and IgG4 Ab for up to 5 years after initiation of SIT and analyzed potential functional implications of their alterations. While we noticed no substantial change of IgE Ab concentrations, allergen-specific IgG and IgG4 serum levels continued to increase over the whole SIT-period of 3 years. However, IgG serum concentrations stopped to rise and started gradually to decline already 6 months after the cessation of SIT, but remained significantly elevated above pre-treatment levels. Since the IgG Ab might compete with IgE for capturing specific allergens we investigated the influence of SIT-induced allergen-specific IgG Ab on IgE binding to allergen, histamine release (HR) from basophils and allergen uptake mediated by the low affinity IgE-receptor CD23 on antigen-presenting cells. Analyzing serum samples obtained from 12 SIT-treated birch pollen allergic individuals during 3 years of active treatment and up to 18 months after ending the therapy, we could demonstrate that SIT-induced increases of IgG and IgG4 Ab concentrations were accompanied by enhanced inhibition of allergen binding to IgE, diminished HR and reduced allergen uptake by B cells. In contrast, the concomitant decrease of IgG and IgG4 Ab concentrations after cessation of SIT was followed by a gradual abrogation of these IgE-blocking effects.

In summary, these data show that allergen-specific IgG Ab induced by SIT might play a crucial role in establishing allergen tolerance by blocking IgE-allergen interactions important for the generation of immediate type allergic reactions. Long-term studies following the post-treatment course of SIT are necessary to determine if IgG Ab concentrations can be maintained high enough to conserve these effects and thus result in persistent allergen tolerance.

P016 (V13)
Protective role of CB1 receptors on keratinocytes in a mouse model of allergic contact dermatitis

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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). We previously reported that the ECS attenuates allergic contact hypersensitivity (CHS) responses in the skin. Further experiments involving adoptive lymphocyte transfer and bone marrow chimeras using mice genetically deficient for both known CB receptors provided evidence that endocannabinods act on radioresistant resident skin cells in the challenge phase of CHS. To further understand the underlying mechanisms we performed detailed time-course analyses of CHS responses in CB1 receptor-deficientmice. Histological analyses and BrdU-proliferation assays revealed an enhanced and prolonged inflammation associated with abnormal keratinocyte proliferation and differentiation. mRNA levels of IL-β and CXCL2, two important activators of keratinocytes, were significantly elevated in inflamed ear tissue of CB1-/- mice compared to wildtype animals. As demonstrated by an increase of transepidermal water loss, CB1 receptor-deficient mice also showed an impaired cutaneous permeability barrier homeostasis. Using conditional knock out mice we found that tissue-specific deletion of the CB1 receptor in keratinocytes but not in sensory neurons largely recapitulates this phenotype. Taken together, our observations reveal a previously unrecognized physiological role of CB1 receptors on keratinocytes for the maintenance of epidermal integrity and permeability barrier functions by endocannabinoids which contributes to the protection against contact allergic inflammation.

P017
Effects of different brands of medical compression stockings (MCS) on skin barrier function, microbial status and wearing comfort of the stocking

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Introduction: MCS are designed to improve musculovenous pump performances due to incompetent leg vein valves. The goal of improved circulation and muscle efficiency is reached by use of various fabrics. Fabrics are known to have an important impacton clinical performance of compression therapy. Therefore we investigated differences of two actual stocking brands by comparing skin barrier function, skin moisture, temperature kinetics and influence on the skin flora.

Methods: Twelve healthy volunteers wore two different stockings (brands A and B) on each leg. The volunteers experienced physical stress on a treadmill. The medical compression stockings were worn while different tests were carried out: skin moisture, transepidermal water loss (TEWL, Courage and Khazaka, Germany) and skin temperature. Additionally microbial investigations of the skin flora and gravimetric measurements of the stockings were performed.

Results: After treadmill stress, wearing group A stockings resulted in significantly higher amounts of transpiration fluid content in the stockings. In the same group physical stress (treadmill) was followed by faint increase of transepidermal water loss in parallel with decreased skin moisture. Additionally in this group a slight increase of the temperature was measured during the physical stress period as well as elevated amounts of bacteria on the skin under the stockings.

Conclusion: Fabrics in medical compression stockings may influence the skin barrier integrity and the surrounding microclimate of the involved leg skin. Changes in fluid management of certain stockings with consecutively elevated volumes retained in the fabrics may act as an occlusive and therefore impede evaporation with the result of increasing temperatures. These differences may contribute to elevated amounts of underlying physiological skin flora.

P018
Influence of medical compression stockings on skin barrier function and haemodynamics at patients suffering from chronic venous insufficiency (CVI)

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Introduction: Skin dryness and itching are most often recorded complaints by patients suffering from CVI wearing medical compression stockings. Skin shear-stress exerted by the stocking fabrics is discussed as one cause of this effect. One solution to overcome this problem could be the impregnation of the stocking fabrics with care products during fabrication or during the washing procedure at home. Therefore we investigated the effects of a new stocking with integrated skin care emulsion on skin barrier function and skin moisture of patients with CVI.

Methods: Seven of 20 patients suffering from longstanding CVI (Widmer I und II) wore commercially available classical (medical) compression stockings (CCS) as control and during a second period a stocking (CCE, as verum) which has to be washed (additionally to daily washing) every 3 days adding a skin care emulsion. Patients wore stockings (blinded, CCS or CCE) 8 h a day over 14 day. Before and after each test period the skin barrier function parameters (roughness, skin moisture and transepidermal water loss) (TEWL, Courage & Khazaka, Germany) were measured together with haemodynamic parameters [refilling time of veins (by DPG), surface pressure and legvolume] after an acclimatization-time of 20 min. The wearing comfort was recorded using a questionnaire every second day. During the whole 6-week-long period patients were not allowed to use other external skin care products on the legs.

Results: Wearing stockings with added skin care emulsion (CCE) in comparison with controls (CCS) was followed by a slight decrease of TEWL instead of a marked increase with controls and a nearly constant skin moisture (instead of strong decrease with CCS).The comfort wearing CCE gave better results than CCS when sweating under the stockings was recorded by questionnaire. The leg volume after wearing CCE showed a weak increase instead of a decrease measured with CCS.

Conclusion: CCE can impair the wearing performance of stockings and counteract the known drying effects by medical compression stockings). Further evaluation to monitor the maintenance of haemodynamic efficacy is mandatory.

P019
Involvement of fibroblasts in adipose tissue development

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Human adult somatic stem cells have come to the centre of scientific attention due to their multipotent abilities, and those of the skin due to easy access. Fibroblasts, cells of mesodermal origin and major structural cells of the dermis, are regarded as almost differentiated cells. Such differentiated cells can still not be the origin for considerable amounts of differentiated adipocytes. It has been reported that a few fibroblasts can differentiate into adipocytes. However, it has been disputed whether these adipocytes derive from fibroblasts or from preadipocytes, a precursor form of adipocytes.
Aim of the Project: To establish a method, which enables obtaining respectable numbers of adipocytes from differentiated human primary fibroblasts of the skin.
Materials and Methods: Primary fibroblasts were obtained from skin specimens of human foreskin and of the light-protected area of the inner upper arm of women 30-and 76-year old. An adipocyte induction system was applied and lipid accumulation in the induced fibroblasts was analysed by fluorescence microscopy, flow cytometric analysis (FACS) and electron microscopy. Lastly, the transcriptional phenotype of the cells was determined and compared with that of control cultures and genuine adipose tissue, a comprehensive group of adipocyte-specific genes was analyzed by a sensitive real-time RT-PCR and microarray gene analysis.
Results: Under special culture conditions, cultured skin fibroblasts are capable to differentiate into adipocytes. This ability is stronger in cells derived from young skin in comparison with those derived from older skin.
Conclusion: Induced fibroblasts and the derived adipocytes could be used forther apeutic needs in regenerative medicine (cell and tissue replacement) as well as in cosmetic dermatology.

P020

Dissecting the molecular mechanisms involved in human endogenous skin ageing in both genders

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The development of genomic tools has facilitated deeper insights into the molecular events underlying human ageing. The identification of pathways affected by skin ageing has led to the development of new strategies to possibly enable skin repair. In the current study, we investigated the gender-specific differences in the expression profiling of aged skin between young and elderly adults. Whole genome gene expression patterns were examined in sun-protected skin obtained from European Caucasian young and elderly males [mean age 20 ± 10 (n = 7) and 60 ± 10 (n = 7), respectively] and females [mean age 20 ± 10 (n = 7) and 60 ± 10 (n = 4), respectively] using the Illumina array platform. Confirmation of gene regulation was performed by real-time RT-PCR. Eight hundred and seventy genes were significantly regulated in female skin and 383 genes in male skin using the chosen criteria. Of these, 286 genes exhibited increased and 584 decreased expression with age in females and 213 genes showed increased and 170 decreased expression in males. Differentially expressed genes were further filtered according to Gene Ontology (GO) terms or mapped to KEGG pathways using DAVID. The functional annotation of these genes identified several pathways related to mitochondrial function, oxidative stress, ubiquitin-mediated proteolysis, cell cycle, organization and adhesion, immune responses, steroid biosynthesis and phospholipid degradation. In total, 43 genes were common in the target lists of significantly regulated genes in males and females. Forty one of these genes showed increased (16) or decreased (25) expression independently from gender. Only two genes showed different regulation with increased expression in male and decreased expression in female skin biopsies. In conclusion, our study provides biomarkers of endogenous skin ageing in both females and males, which are not dependent on gender, and therefore, on the different hormone status.

P021

Age-related skin changes on the molecular level

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Background: Ageing is a complex process that also involves the decline or dysfunction of the skin. The corticotropin-releasing hormone (CRH) system, which is involved in inflammatory skin diseases, peroxisome proliferator-activated receptors (PPAR), known to play a critical role in tumorigenesis and metabolism, inflammatory cytokines and the Toll-like receptor (TLR)-4, which is involved in innate immunity and chronic inflammation, have been shown to be affected in several age-associated conditions and in animal ageing models. Therefore, a systematic study was conducted to detect the expression of these molecules during the healthy skin ageing process.
Methods: The expression of CRH, CRH-binding protein (CRH-BP), CRH receptor (CRH-R) 1 and 2, PPAR-α, -δ and -γ, TLR-4 and the inflammatory cytokines interleukin (IL)-6 and -8 in 44 healthy skin samples of individuals in different ages (4–92 years) was evaluated by immunohistochemistry.
Results: Compared with young skin, the ageing skin displayed an age-dependent up-regulation of CRH in sebaceous glands, CRH-BP in the dermis, CRH-R1 in hair follicles and epidermis and IL-6 in the dermis, while it showed age-dependent down-regulation of CRH in the sweat glands, CRH-BP in the sebaceous and sweat glands, PPAR-α and TLR-4 in all skin compartments (epidermis, hair follicles, sebaceous and sweat glands), PPAR-γ in the sebaceous glands, IL-6 and IL-8 in sweat and sebaceous glands.
Conclusion: Our findings suggest that the age-dependent changes in the expression of CRH system components, PPAR, TLR-4 and inflammatory cytokines could partly explain the ageing process including tumorigenesis, defective stress response reaction and defective immune response. An interaction between the CRH system, inflammatory molecules and PPAR was evident.

P022

Generation of induced pluripotent stem cells from healthy and patient-derived human skin biopsies as model for *in vitro* investigation of systemic diseases

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We and others have previously shown that genes and gene pathways associated with healthy intrinsic ageing are markedly common with those associated with systemic diseases. Induced pluripotent stem cells (iPS) offer crucial potential for disease research, drug screening, toxicology and regenerative medicine. However, little is known about the nature and sequence of molecular events accompanying nuclear reprogramming. In order to further investigate the detected skin-system organs association, we administered cell infection with retroviruses expressing the four transcriptional factors OCT4, SOX2, KLF4 and C-MYC and we generated iPS cells from skin-derived fibroblasts obtained from a healthy neonate (BJ, HFF1, purchased from ATCC, LGC Starndards, Wesel, Germany) and from an 84 year-old patient diagnosed to suffer from type II diabetes (NFH-2). The iPS colonies, which formed 4 weeks after infection exhibited the essential characteristics of embryonic stem (ES) cells, such as alkaline phosphatase (AP) activity, expression of cell surface markers and pluripotency-associated genes. In order to control the maintainance of the molecular identity of the generated iPS cells, we analysed the parental somatic cells and their corresponding iPS cells by molecular parameters with regard to apoptosis, LDH, proliferation rate, HLA, telomerase activity, telomere length measurement and formation of ROS. Comparative analysis between NFH-2, BJ, HFF-1 and their iPS cells is in progress. In summary, the aim of the project is to produce systemic organ cells from iPS cells *in vitro* as a model to investigate the molecular pathogenesis of systemic diseases.

P023

Zileuton prevents the activation of the leukotriene pathway and reduces sebaceous lipogenesis

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Arachidonic acid (AA) activates the 5-lipoxygenase, induces leukotriene-B4 (LTB4) synthesis, enhances interleukin-6 (IL-6) release and increases intracellular neutral lipids in human sebocytes. Moreover, the enzymes of LTB4 biosynthesis are activated in acne-involved sebaceous glands. Zileuton, a 5-lipoxygenase inhibitor, reduces the number of inflammatory acne lesions and lipogenesis in patients with acne. In this study, we investigated the activity of zileuton on LTB4 generation, lipid content and IL-6 and -8 release from human SZ95 sebocytes *in vitro*. Pretreatment with zileuton partially prevented the AA-induced LTB4 and IL-6 release and increased neutral lipid content. IL-6 release and neutral lipid content were also reduced under long-term zileuton treatment. In conclusion, zileuton prevents the activation of the leukotriene pathway and enhancement of lipogenesis by AA inhuman sebocytes *in vitro*.

P024

Biomarkers for Adamantiades-Behet's disease

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Purpose of the study was to detect serological predictive course-parameters in Adamantiades-Behet's disease (ABD). Serum/blood of 122 ABD patients inactive/inactive stages and of 75 controls was screened for IL1α, IL1β, IL8, TNFα, sICAM1, bFGF, CRP, ESR. Serum IL18, IL8, MIP1α and CRP and ESR were investigated prospectively in another 18 ABD patients (11 with disease exacerbations in 2 years) and 16 controls. Increased IL8 serum levels, but not IL1α, IL1β, IL6, TNFα, sICAM1, bFGF or ESR were found in active compared with inactive ABD patients and controls. Patients with oral aphthae and neurological features presented higher IL8 levels. An association of IL8 levels with the number of active clinical signs and the presence of severe oral aphthae was detected. Patients having systemic symptoms along with oral aphthae had significantly higher CRP and VEGF levels during the symptomatic period. In the longitudinal prospective evaluation, IL18 was increased in active and decreased in inactive periods in the individual patient's course. IL18 was elevated even in the presence of few oral aphthae or mild uveitis. Comparably, CRP was a useful course-indicator, while MIP1α and ESR showed no reliable correlation with disease activity. IL18 is the most sensitive, single sign-parameter correlating with disease activity in ABD, followed by IL8, which increases with the number of clinical signs. CRP and VEGF can also be used as biomarkers to identify systemic but not mucocutaneous disease. The combination of these four serological parameters can be useful in the follow-up of ABD patients, especially in therapeutic trials.

P025

The sebocyte-own corticotropin-releasing hormone system is an amplifier of inflammation

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Activation of the hypothalamic-pituitary-adrenal axis, which is the main adaptive response to chronic systemic stress, requires production of corticotropin-releasing hormone (CRH). CRH has also been detected in peripheral tissues, including the skin. Human sebaceous glands and cultured human SZ95 sebocytes express CRH, CRH-binding protein (CRH-BP) and CRH receptors (CRH-R) 1 and 2 at the mRNA and protein levels as we have shown by RT-PCR and immunohisto- and immunocytochemistry. CRH and urocortin, a peptide which shares a 45% homology with CRH, inhibit SZ95 sebocyte proliferation, which can be restored byα-helical-CRF, a CRH antagonist. CRH upregulates 3β-hydroxysteroiddehydrogenase/Δ5-4 isomerase mRNA levels and testosterone accordingly downregulates CRH-R1 and CRH-R2 mRNA expression in a negative feedback manner. Moreover, CRH enhances the basal synthesis, while antalarmin, anon-peptidic selective CRH-R1 antagonist, reduces the increased production of neutral lipids in SZ95 sebocytes. CRH also stimulates interleukin (IL)-6 and IL-8 release from SZ95 sebocytes but has no effect on IL-1α and IL-1β production or theIL1α-induced IL-8 release in these cells. Inflammation, such as acne, and proinflammatory signalling, such as UVB or the bacterial antigen MALP-2 amplify intracellular CRH levels, while dexamethasone inhibits the intracellular CRH synthesis in SZ95 sebocytes. No CRH release could be detected; CRH-BP expression was amplified in differentiating sebocytes of acne-involved skin. In conclusion, inflammatory signals can enhance intracellular CRH levels in human sebocytes leading to CRH-R1-associated lipogenesis and amplification of inflammation by secretion of proinflammatory cytokines. These events are sensitive to dexamethasone or specific CRH-R1 antagonists.

P026

The role of the chemokine CXCL1 in epithelialization, inflammation and ageing

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Chronic inflammation and delayed re-epithelialization contribute to the aged-associated impaired wound healing. Furthermore, dysregulation of the inflammatory state is an underlying basis of tissue ageing itself. Sex hormones have been suggested to regulate immune parameters and are essential modulators of skin ageing and several skin tasks, such as wound healing. The chemokine CXCL1/Gro-α (growth-regulated protein-α) is a hormone-regulated molecule and has been involved in growth regu-

lation, angiogenesis, tumorigenesis, wound healing, inflammation, molecular senescence and ageing. To elucidate the role of CXCL1 in senescence, inflammation and epithelialization, human primary epidermal foreskin keratinocytes were transiently transfected at third and fifth passage with CXCL1-short interfering RNA (siRNA) molecules (0.5 nM, AtuRNAi®, Silence, Quark Pharmaceuticals, Freemont, CA, USA) in the presence of *in vivo*-tested cationic lipids (1 μg/ml, AtuPLEX®, Silence, Quark Pharmaceuticals, Freemont, CA, USA). The protein knock-down of CXCL1 and the levels of the pro-inflammatory cytokine IL-8 were assessed by ELISA. The corresponding RNA expression of CXCL1 was determined by RT-PCR. The mobility and proliferation rates of the native and CXCL1-transfected keratinocytes were measured by *in vitro* scratch and proliferation assays (XTT). The optimal inhibition of the expression of CXCL1 protein was at passage 3 about 55% and at passage 5 about 20% and on RNA level about 70% without major differences between passage number compared to exclusively AtuPLEX®-treated controls. Furthermore transfection with CXCL1 siRNA slightly reduced IL-8 protein expression but did not affect keratinocyte proliferation and mobility rates. In conclusion, CXCL1 protein and RNA expression can be controlled by the specific siRNA technology and the protein down-regulation depends on the time in passage. Further studies have to be done to quantify the effects of the molecule on other inflammatory mediators.

P027

Exonuclease-1 exhibits regulation in apoptosis and proliferation under hormone-depending conditions in human fibroblasts

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Introduction: Exonuclease-1 (EXO1) is a member of the RAD2 family of structure-specific nucleases and has 5′ to 3′ exonuclease as well as RNase H activity. Genetic analysis has identified roles for EXO1 in mismatch repair, replication, recombination, DNA repair and maintenance of telomeres, which play a significant role in the generation of ageing. Furthermore, EXO1 has been shown to be differentially expressed in human SZ95 sebocytes treated with a hormone mixture consisting of growth factors and sex steroids at concentrations corresponding to those circulating in 20- (f20) and 60- (f60) year old females.

Materials and Methods: The expression of EXO1 was tested in SZ95 sebocytes, primary fibroblasts (foreskin, breast), DFs fibroblasts and 3T3 cells, maintained under hormone-substituted conditions at mRNA and protein levels via real-time PCR and western blotting, respectively. Furthermore, the cells were transiently transfected with EXO1 siRNA in the presence of cationic lipids and synthetic ribo oligonucleotides (nMarea, both from QIAGEN, Hilden, Germany). After transfection, the cells had been tested for proliferation, cytotoxicity and apoptosis. The confirmation of the gene regulation was also performed by means of real-time PCR.

Results: EXO1 was found to be expressed in human SZ95 sebocytes, primary fibroblasts and 3T3 cells. EXO1 was significantly up-regulated in human SZ95 sebocytes at mRNA and protein levels in the cells at f60 hormone levels. Furthermore, the expression of EXO1 was significantly inhibited via RNA interference at mRNA in SZ95, fibroblasts and 3T3 cells (50–80%) and protein levels in SZ95 (66%, $P < 0.001$). EXO1 also seems to be inhibiting proliferation and apoptosis at f20 hormone concentrations.

Conclusion: The evaluation of age-associated genes via RNA interference could facilitate our understanding on the molecular mechanisms involved in ageing. Our data imply that EXO1 may be a hormone-dependent gene and may be implicated in the impairment of the DNA repair accompanying the ageing process.

P028

K(D)PT, a tripeptide related to the C-terminal sequence of α-melanocyte-stimulating hormone, efficiently ameliorates ongoing psoriasis in a humanized mouse model

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The proopiomelanocortin derived tridecapetide α-MSH as well as its C-terminal tripeptide KPV are known to exhibit potent anti-inflammatory and immunomodulatory effects *in vitro* and *in vivo*. Recently, K(D)PT a tripeptide derived from an inhibitory loop of IL-1β and structurally similar to KPV but characterized by an increased stability and safety was shown to have identical anti-inflammatory and immunomodulatory effects compared to KPV and α-MSH. Of note, the anti-inflammatory effects of K(D)PT might be mediated by a reduction of nuclear factor κB (NF-κB) activation, a transcription factor which is crucial for the regulation of inflammatory processes. Efficacy of K(D)PT has previously been demonstrated in mouse models of inflammatory bowel disease and contact allergy. In this study we analyzed in a humanized mouse model whether K(D)PT is able to ameliorate ongoing psoriasis. Therefore, full-thickness skin biopsies from non-lesional skin of patients with moderate to severe psoriasis were transplanted onto beige nude XID (BNX) mutant mice. Three weeks after skin transplantation activated peripheral blood mononuclear cells (PBMCs) isolated from the same patients were injected into the transplants resulting in the development of severe psoriatic lesions. Seven days later recipient mice were treated for 3 weeks with PBS (100 l, i.p.), 1 g K(D)PT (in 100l PBS, i.p.), 10 g K(D)PT (in 100 l PBS, i.p.) or betamethasone dipropionate as a positive control (15 mg, topical). Interestingly, K(D)PT treatment resulted in a dose dependent, significant reduction of epidermal ridge thickness and moreover, the keratinocyte proliferation index was dramatically reduced compared to the PBS treated control mice as evidenced by cytokeratin 16 and Ki-67 staining. Notably, incontrast to the betamethasone dipropionate treated mice, recipients injected with K(D)PT did not show body weight loss. To verify these data in a second mouse model for psoriasis we induced a psoriasis-like skin inflammation in Balb/c mice bytopical application of imiquimod (Aldara®, Meda Pharma, Bad Homburg, Germany) and injected the mice with PBS (100 l, i.v.), 5 g K(D)PT (in 100 l PBS, i.v.) or anti-TNF-α as a positive control (5 g in 100 lPBS, i.v.). Strikingly, K(D)PT treatment resulted in a significant reduction of epidermal thickness and furthermore, decreased the levels of pathogenic Th17 cells in lesional skin as well as regional lymph nodes dramatically. Since it has been shown that the numbers of pathogenic Th17 cells are increased in peripheral blood from psoriasis patients compared to healthy controls we analyzed whether K(D)PT might affect human Th17 cells. Therefore, PBMCs from patients with moderate to severe psoriasis were stimulated with K(D)PT and interestingly, K(D)PT resulted in a significant reduction of total Th17 cells and moreover, decreased the secretion of pro-inflammatory cytokines in these cells. Together, our results indicate that the tripeptide K(D)PT is able to ameliorate ongoing psoriasis and suggest that K(D)PT might represent a potential therapeutic option for the treatment of patients with moderate to severe psoriasis.

P029

Pregnane X Receptor (PXR) links xenobiotic metabolism to the cutaneous immune response

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The pregnane X receptor (PXR) is a ligand-activated transcription factor regulating genes central to drug and hormone metabolism in the liver. We have recently shown that ligand activation of PXR suppresses T-lymphocyte function. We here show that topical applications of PCN, a well-known activator of mouse PXR, ameliorates irritant and allergic contact dermatitis in a PXR-dependent manner. Moreover, rifampicin, an activator of the human PXR, when applied topically improves allergic contact dermatitis in mice humanized for PXR and for both PXR and CYP3A4, an important PXR down-stream gene. Rifampicin down-regulates the expression ofIL-1β in PXR-humanized keratinoctyes in an antigen-specific and receptor-mediated manner. Conversely, PXR deficient mice exhibit exaggerated irritant and allergic contact dermatitis. Increased ear swelling is associated with inflammatory infiltrates mainly consisting of CD4+ T-lymphocytes. Adoptive transfer experiments demonstrate that T-lymphocytes *per se* are not responsible for the pro-inflammatory phenotype in PXR deficient mice, suggesting synergy with other cell types, potentially keratinocytes. Furthermore, PXR is expressed in lymphocyte-rich infiltrates and basal keratinocytes of various human inflammatory skin diseases. In conclusion, PXR links xenobiotic metabolism to the skin immune response.

P030

Vitamin D treatment suppresses the Th17-induced proinflammatory S100 alarmins psoriasin (S100A7) and koebnerisin (S100A15) in psoriasis

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The antimicrobial proteins of the psorias in (S100A7) and koebnerisin (S100A15) are upregualted with inflammation. Although highly homologous, their distinct expression and functions suggest a synergistic role as antimicrobials and proinflammatory' alarmins' in the pathogenesis of chronic inflammatory psoriasis. However, the mechanism of their regulation in psoriasis is not known and how they are affected by anti-psoriatic therapy. Th1/Th17 cytokines are key players in the pathogenesis of psoriasis and vitamin D analogs have antagonizing anti-psoriatic effects that could be mediated through 'alarmin' regulation. We found that supernatants of T cell isolated from lesional psoriasis induced S100 in keratinocytes signaled through IL17R. *In vitro*, IL17A is principal inducer of psoriasin/koebnerisin in keratinocytes and potentiated their expression with T cell-derived Th1/Th17 cytokines. In return, psoriasin and koebnerisin prime epidermal keratinocytes for enhanced production of immunotropic cytokines, such as TNFalpha and IL8, suggesting S100A7/S100A15 being involved in the autocrine amplification loop of the cutaneous inflammation. Treatment of psoriatic plaques with the topical vitamin D (1,25D3) analogue calcipotriol suppressed the expression of proinflammatory psoriasin and koebnerisinin psoriatic skin. Antipsoriatic vitamin D analogs further interfered with the S100 induction by supernatants of T cell isolated from lesional psoriasis and with stimulation by Th1/Th17 cytokines in epidermal keratinocytes. Data suggests that suppression of proinflammatory alarmins in keratinocytes participates in the anti-inflammatory effect of vitamin D analogs. Thus, targeting the S100-regulatingsystem could be beneficial in psoriasis and other inflammatory skin diseases.

P031 (V09)

Small S100 molecules with cutaneous antimicrobial activity prime skin for inflammation: a mouse model for psoriasis susceptibility

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Psoriasis is genetically linked to small molecules of the human S100A7/A15 subfamily encoded within the psoriasis susceptibility locus at chromosome 1q21.Inflammation-prone psoriatic skin is characterized by constitutively elevated levels ofS100A7/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 a pathogenetic model for inflammation priming. Thus, targeting S100A7A15-RAGE may be a novel therapeutic approach for treatment of susceptibility and inflammation in psoriasis.

P032

Alpha-MSH inhibits TNF-alpha-mediated responses in human melanocytes possibly by induction of suppressors of cytokine signaling 1/3

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Suppressors of cytokine signaling (SOCS) are proteins which negatively regulate cytokine signaling. Several cytokines such as interleukin (IL)-1, IL-6 or tumor necrosis factor-alpha (TNF-alpha) are typically induced after UVB irradiation of epidermal cells and suppress melanogenesis and proliferation of normal human melanocytes (NHM).On the other hand, UVB exposure induces alpha-melanocyte-stimulating hormone (alpha-MSH) which promotes melanogenesis and proliferation of these cells. During the UVB response the melanocyte therefore receives antagonistic signals. We hypothesized that alpha-MSH, by induction of SOCS1/3, orchestrates the response of NHM to the aforementioned cytokines. First, we examined if alpha-MSH directly affects SOCS1/3 expression. SOCS1/3 mRNA expression was time- and dose-dependently induced after alpha-MSH treatment of NHM. Western immunoblotting confirmed the upregulating effect of alpha-MSH on SOCS3.Preincubation with actinomycin D disclosed transcriptional regulation of the SOCS1/3 genes by alpha-MSH. Pharmacological inhibition studies further revealed that theSOCS1/3-inducing effect of alpha-MSH is dependent on both the cAMP-PKA and the MAPK-MEK pathway. Moreover, the promoter regions of both SOCS1 and SOCS3 contained putative CREB binding sites as shown by *in silico* promoter analysis. Accordingly, artificial cAMP inducers mimicked the upregulating effect of alpha-MSH on SOCS1/3 mRNA expression. Preincubation of NHM with alpha-MSH suppressed TNF-alpha-induced nuclear translocation of NF-kappaB/p65 and IkappaBalpha degradation in line with the previously reported molecular interference of SOCS1/3 with p65. In addition, preincubated alpha-MSH suppressed TNF-alpha-mediated IL-6 and IL-8 mRNA induction and antagonized the TNF-alpha-mediated suppression of melano-

genesis key enzymes. Our findings show that SOCS1/3 expression is turned on by alpha-MSH in melanocytes. Via induction of SOCS1/3, alpha-MSH may orchestrate the behaviour of epidermal melanocytes after UVB irradiation towards a protective response.

P033
Superoxide dismutase 2 – a putative mediator of the antifibrotic effect of alpha-MSH in the bleomycin model of scleroderma

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Identification of novel treatments for fibrosclerotic diseases remains a major therapeutic challenge in medicine. Using the well established bleomycin (BLM) model for scleroderma we previously reported that alpha-melanocyte-stimulating hormone (alpha-MSH) attenuates collagen synthesis *in vitro* and *in vivo*. However, the mechanism by which alpha-MSH exerts its effects is still incompletely understood. Since anti-oxidative enzymes play an important role in fibrosis including the BLM model of scleroderma we investigated in detail the molecular regulation of superoxide dismutase (SOD) in human dermal fibroblasts (HDF) by alpha-MSH. Alpha-MSH time- and dose-dependently induced the mRNA expression of SOD2 but not of SOD1 and SOD3. This alpha-MSH-mediated effect was confirmed at protein level by Western immunoblotting. Accordingly, total SOD enzyme activity was increased by 45% after incubation with alpha-MSH for 24 h. Next, we determined the molecular mechanism by which alpha-MSH induces SOD2 expression. The effect of alpha-MSH on SOD2 in HDF was melanocortin-1 receptor-mediated as demonstrated by preincubation with a truncated but functional peptide corresponding to agouti signaling protein. In accordance with this MC-1R transfected 3T3 murine fibroblasts but not vector-alone transfected cells showed an induction of SOD activity upon stimulation with α-MSH. Using the RNA polymerase blocker actinomycin D, we found that alpha-MSH transcriptionally induces the SOD2 gene. Artificial cAMP inducers mimicked the effect while an adenylate cyclase inhibitor neutralized the effect of alpha-MSH on SOD2 mRNA expression. In support of an cAMP-mediated mechanism *in silico* promoter analysis revealed several CREB bindings sites in the human SOD2 promoter. Knockdown experiments of SOD2 are currently under way to definitively show that alpha-MSH via SOD2 mediates its suppressive effect on BLM-induced collagen synthesis and fibrosis.

P034
Epigenetic modulation of vitamin D signaling pathways in angiosarcoma cells *in vitro*

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Recent investigations convincingly demonstrate that the vitamin D endocrine system (VDES) is implicated in various tissues in cellular differentiation, apoptosis, tumor development and progression. Modulation of vitamin D signalling pathways is therefore considered as a promising new potential target for cancer prevention and treatment. Angiosarcoma is a rare tumor entity that remains difficult to treat. However, little is known about the expression and function of key components of the VDES in angiosarcoma cells. Using real time PCR (Light Cycler, Rocher, Mannheim, Germany) and western analysis, we have therefore now characterized for the first time expression of key components of the VDES in AS7B cells, one of the seldom angiosarcoma cell lines expressing VEGF165. We demonstrate that vitamin D receptor (VDR), 25-hydroxyvitamin D-1alpha-hydroxylase (CYP27B1) and vitamin D-24-hydroxylase (CYP24A1) are strongly expressed in AS7B cells. In the next step, we proved that the biologically active vitamin D metabolite 1,25-dihydroxyvitamin D3 exerts a slight but measurable antiproliferative effect on AS7B cells. Moreover, we were able to show that epigenetic modulator drugs trichostatin A (TSA, inhibitor of histone deacetylases) and 5-azacytidine (5-AZA, inhibitor of DNA methyl transferases) amplify the antiproliferative effect of 1,25-dihydroxyvitamin D3 on AS7B cells. In conclusion, our findings support the concept that modulation of vitamin D signaling pathways represents a promising new target for the prevention and therapy of angiosarcoma.

P035
An *in vitro* test system to study human epidermal wound healing in situ

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Chronic skin ulcers are a major medical problem for which more satisfactory treatment urgently needs to be developed. We have therefore developed a standardized full-thickness human skin wound healing assay that permits the study of cutaneous regeneration in situ. The absence of systemic effects in this preclinical invitro model also resembles the situation in chronic skin ulcers which are based on or aggravated by insufficient perfusion. Several quantifiable parameters for regenerative epidermal processes were measured: Ki67/TUNEL for proliferation and apoptosis, MTCO1 for energy metabolism, K6 for activated keratinocytes and MSX2 and involucrin for early and late stages of keratinocyte differentiation, respectively. We also measured the effects on local inflammatory responses and on vascularisation by counting the number of mast cells and CD31-positive endothelial cells in the dermis. Several wound healing-promoting candidate substances were tested: serum from several species (human, bovine, frog), thyroid hormones, the neuropeptides TRH, bombesin, neuromedin B (NMB) and gastrin releasing peptide (GRP), and estradiol. All of these substances led to a significant increase of epithelial outgrowths at the wound edges compared to vehicle-treated samples. Interestingly, the effects on the epidermal and dermal wound healing parameters were quite distinct: TRH, thyroxin and estradiol led to significantly higher numbers of Ki67-positive cells and MTCO1 immunoreactivity, whereas the peptides of the bombesin family increased the number of endothelial cells. This effect was likely mediated by upregulaton of the receptors for GRP and NMB. Treatment with TRH also significantly increased the number of histochemically detectable mast cells in the wounded dermis.

With this set of quantitative parameters it was possible to further dissect the molecular mechanisms of these substances on distinct processes during cutaneous wound healing. Therefore this assay provides a valuable preclinical tool for identifying the reepithelialisation effects and molecular signature of candidate wound healing promoters.

P036 (V30)
Challenge and promise: epigenetic modulation of antiproliferative effects of 1,25(OH)2D3 in malignant melanoma cells *in vitro*

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Melanoma cells express the vitamin D receptor (VDR), indicating that malignant melanoma may represent a promising target for treatment with vitamin D analogs. We have previously analyzed in seven different melanoma cell lines the antiproliferative effects of the biologically active vitamin D metabolite1,25-dihydroxyvitamin D3 [1,25(OH)2D3]. While three cell lines (MeWo, SK-Mel28, SM) responded to antiproliferative effects of 1,25(OH)2D3, the others (SK-Mel5, SK-Mel25, IGR, MelJuso) were resistant. We have now investigated whether combination of 1,25(OH)2D3 with epigenetic modulating drugs may represent a promising tool to overcome the resistance against the antiproliferative effects of 1,25(OH)2D3 in melanoma cells. We used a combination of 1,25(OH)2D3 and the histone deacetylase inhibitor (HDACI) trichostatin A (TSA) to elucidate the effect of histone acetylation on 1,25(OH)2D3-sensitivity. Additionally, we studied the antiproliferative effect of 1,25(OH)2D3 in combination with 5-azacytidine (5-Aza), a DNA methyltransferase inhibitor (DNMTI), to investigate the putative effects of DNA methylation. Interestingly, additive antiproliferative effects were found after treatment with 1,25(OH)2D3 (10–8 M) in combination with TSA (15 ng/ml) in 1,25(OH)2D3-resistant cell lines and after treatment with 1,25(OH)2D3 (10–8 M) in combination with 5-Aza (10 M) in 1,25(OH)2D3-resistant and -responsive cell lines. To gain further insights in the epigenetic modulation of vitamin D signaling in melanoma, we studied the expression of two candidates of VDR microRNAs (miR-125b and miR-27b) in 1,25(OH)2D3-responsive and -resistant melanoma cell lines. Interestingly, VDR mRNA expression was relatively higher in 1,25(OH)2D3-responsive as compared to 1,25(OH)2D3-resistant cell lines, while incontrast, VDR microRNA (miR-125b) expression level was relatively higher in 1,25(OH)2D3-resistant as compared to 1,25(OH)2D3-responsive melanoma cell lines. Taken together, our findings indicate that responsiveness of melanoma cells against the antiproliferative effects of 1,25(OH)2D3 corresponds to the expression level of VDR mRNA, that may be regulated by expression of VDR microRNAs (miR-125b and miR-27b). Moreover, our findings suggest that epigenetic modulating drugs modulate vitamin D signaling in melanoma cells and may represent a promising tool to overcome the resistance against antiproliferative effects of vitamin D analogs.

P037
Tonic inhibitory effects of endocannabinoids on human skin mast cellfunctions in situ by cannabinoid receptor 1 (CB1)-mediated signalling

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Since many chronic inflammatory and allergic skin disorders are characterized by excessive mast cell (MC) numbers and activation, it is clinically important to understand the physiological controls that avoid increased MCnumbers/degranulation in normal human skin. Recently, cannabinoids have surfaced as a potentially important class of neuroendocrine regulators in MC biology. We have previously shown that locally produced prototypic endocannabinoids (e.g. anandamide, AEA) markedly inhibit the growth of organ cultured human hair follicles (HFs) via cannabinoid receptor (CB) 1 (Telek *et al*, FASEB J 2007). Since perifollicular MCs are important regulators of murine hair growth, we have now investigated the effects of CB1-signalling on normal human skin MCs in situ, focusing on MCs in the connective tissue sheath (CTS) of organ-cultured human scalp HFs.

Here, we show that Kit+ CTS MCs express functional CB1 receptors in situ. Blockade of CB1-signalling (using the specific CB1 antagonist AM251 or CB1 gene knockdown by siRNA) significantly enhanced MC degranulation, as shown by MC histochemistry, tryptase immunohistochemistry and ultrastructurally. Strikingly, the inhibition of CB1-mediated signalling also promoted MC maturation from resident progenitor cells in the CTS, and significantly increased the number of Kit+, FcεRI+, tryptase+ and chymase+ CTS cells, probably via up-regulating stem cell factor (SCF) transcription and protein production by the HF epithelium. Similar MC phenomena were seen inCB1 knock-out mice. In contrast, both the CB1 selective agonist, ACEA, and the endocannabinoid, AEA, counteracted the MC-activating effects of potent endogenous and exogenous MC secretagogues (substance P, compound 48/80) in situ.

These data provide the first evidence that normal human skin MCs are subject to an important inhibitory endocannabinoid tone that controls MC maturation from resident progenitors and thus the number of differentiated MCs, as well as MC activation. This newly identified 'natural clamp on excessive MC activities' may serve as an important future target in the management of allergic diseases. Furthermore, we show that the CTS of human HFs offers an excellent, physiologically and clinically relevant model system for investigating and manipulating the biology of human skin MCs within their natural tissue context habitat.

P038
Vaspin and psoriasis

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Vaspin (Visceral adipose tissue-derived serine protease inhibitor), is a member of the serine protease inhibitor family related to obesity and glucose metabolism with mRNA expression in adipose tissue, liver, pancreas and in skin. The dysregulation of skin proteases and their inhibitors can contribute to the onset of inflammatory skin diseases.

In the present study we investigated the role of vaspin in the pathogenesis of psoriasis, an obesity associated, chronic inflammatory skin disease.

The effect of vaspin on the function of dendritic cells involved in the inflammatory process was analyzed by flow cytometry and ELISA. Vaspin did not influence the differentiation and maturation of monocyte-derived dendritic cells (MoDC) so far.

Furthermore we analyzed the expression pattern of vaspin in skin by immunehistological staining. We detected vaspin expression by keratinocytes in the epidermal layers of the skin in both healthy subjects and psoriasis patients.

To investigate a potential link between vaspin, obesity and psoriasis we measured the vaspin serum level of psoriasis patients and healthy subjects. Vaspin level in serum was elevated in patients with psoriasis and normal BMI. In healthy subjects the vaspin serum level correlated with BMI. In obese subjects the vaspin levels were elevated in both healthy subjects and psoriasis patients.

These results indicate that vaspin is expressed in the epidermis and that the serum level of vaspin is triggered by two different factors, an increased BMI and psoriasis. Further investigations are needed to asses the relation of vaspin serum level and the psoriasis severity and area index and to analyze the regulation of vaspin expression in skin specific cell types.

P039
Prohormone convertases – novel players in melanoma biology

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Proprotein or prohormone convertases (PCs) are Ca2+-dependent serine proteases which do not only process prohormones into biologically active peptides but also activate cancer-related proteins such as growth factors, growth factor receptors, adhesion molecules and matrix metalloproteases.

Although it has previously been reported that some PCs are overexpressed in a number of solid tumors little is known on the role of PCs in melanoma. In order to clarify if PCs are involved in the pathogenesis of melanoma we focused here on subtilisin-kexin isozyme-1 (SKI-1)/proprotein convertase site 1 protease (S1P), PC5/6 and PC7. We first investigated the expression and regulation of these enzymes in normal and transformed human melanocytes. Constitutive expression of SKI-1 atmRNA and protein level was detected in normal human melanocytes (NHM) as well as in nine human melanoma cell lines. PC5/6 expression at the mRNA level was up to 125-fold higher in melanoma cells than in NHM. In contrast, PC7 mRNA levels were significantly lower in all tested melanoma cell lines compared with NHM. In the majority of tested melanoma cell lines PC5/6 protein expression was also higher than in NHM. Immunofluorescence analysis further disclosed a cytoplasmic localization of PC5/6 in melanoma cells. *In silico* promoter analysis of the promotor regions of SKI-1, PC5/6 and PC7 revealed several transcription factor binding sites including those for cAMP response element-binding protein, activator protein-1, sterol-regulatory-element binding protein, activating transcription factor 6 and nuclear factor-kappa B. However, among several growth factors and stimuli tested onlyphorbol-12-myristate-13-acetate modulated the expression of SKI-1 and PC7 but notPC5/6 in NHM. Interestingly, treatment with decanoyl (dec)-RRLL-chloromethylketone (CMK), a pharmacological SKI-1 inhibitor, resulted in a dose-dependent inhibition of the metabolic activity and proliferation of melanoma cells *in vitro*. This effect of dec-RRLL-CMK was associated with reduced expression of caveolin-1, a component of lipid rafts and a tumor promoting gene regulated by sterol-regulatory-element-binding protein-2 which is processed by active SKI-1. In summary, our findings provide evidence for expression and regulation of several new PCs, i. e. SKI-1, PC5/6 and PC7, in human melanocytes. Altered expression ofPC5/6 and PC7 in transformed melanocytes may indicate a pathogenetic role in melanoma. Moreover, pharmacological inhibition of PCs could point towards a novel avenue for the future treatment of melanomas.

P040 (V11)
The proprotein convertase PACE4 mediates increased proliferation, migration and invasiveness of melanoma cells *in vitro* and enhanced subcutaneous tumor growth *in vivo*

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There is accumulating evidence that proprotein/prohormone convertases (PCs) play an important role in the pathogenesis of some solid tumors. Recently, it was also reported that *in vitro* migration and invasion of the human M10 melanoma cell line can be inhibited by the non-selective PC inhibitor alpha1-PDX even in presence of N-Ras, CDKN2A and p53 gene mutations.

Here we investigated the *in vitro* and *in vivo* effects of selective overexpression of the PC member Paired basic Amino-acid-Cleaving Enzyme 4 (PACE4) in melanoma cells. SK-Mel30 melanoma cells expressing low amounts of endogenous PACE4 were stably transfected with rPACE4. *In vitro*, PACE4 transfectants secreted high amounts of rPACE4, showed elevated expression of matrix metalloproteinase 1 and 2, displayed increased cell motility as shown by digital holographic microscopy in collagen as well as enhanced invasiveness demonstrated in Matrigel migration assays. Interestingly, PACE4 transfectants but not vector-alone transfected melanoma cells exhibited also increased metabolic activity and cell proliferation but did not show any change in melanin content. *In vivo*, subcutaneous injection ofPACE4 transfectants into immunodeficient SCID Hairless Outbred mice resulted insignificantly increased local tumor growth compared with injected control cells. However, ectopic expression did not enable transfectants to metastasize.

To finally assess the relevance of these findings we examined the endogenous expression of PACE4 in human melanoma cell lines derived from different stages of disease as well as in human melanoma specimens ex vivo. In six out of nine melanoma cell lines, mRNA expression levels were significantly elevated compared with normal human melanocytes. Immunohistochemical analysis further revealed that PACE4 expression is detectable within melanoma cells in 89% of 47 tumor samples with the highest immunoreactivity in primary melanomas.

In summary, our findings highlight PACE4 as a novel player in melanoma biology and point towards novel future strategies for treatment of melanoma. As local invasion properties are enhanced by PACE4 expression we attribute its function to the early phase of melanoma progression. Further studies have to evaluate in what extent inhibition of PACE4 can reduce tumor growth and invasion.

P041
Neuroendocrine control of mitochondrial function in human epidermis and hair follicles

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It has partly been shown that members of the hypothalamic-pituitary-thyroid (HPT) axis and their corresponding receptors are expressed in human epidermis and the hair follicle.

We have already found that thyrotropin (TSH) and thyrotropin-releasing hormone (TRH) potently stimulate mitochondrial function in human epidermis.

Here we have asked whether TSH and TRH increase mitochondrial activity and biogenesis in human hair follicles as well and if thyroid hormones also influence mitochondria in human epidermis and hair follicles as they are known to stimulate mitochondrial capacity and metabolic potential in many other tissues.

Hair follicles consume energy when growing, epidermis needs ATP to differentiate and proliferate in its basal layers. Thus, it is essential to know how the energy-producing organelles- mitochondria- can be influenced by hormones.

Organ-cultured human skin was treated with T3 (100 pM) or T4 (100 nM) for 24 h. While this did not alter epidermal morphology, both T3 and T4 significantly increase dimmunoreactivity for the mitochondria-selective subunit I of respiratory chain complex IV (MTCO1) and the mitochondrial transcription factor (TFAM) in human skin in situ. Since this suggested an up-regulation of mitochondrial biogenesis, transmission electron microscopy was performed. This revealed an increased number of perinuclear mitochondria in individual keratinocytes in T3/T4-treated epidermis. T3 and T4 also significantly enhanced mitochondrial complex I activity as shown by NBT reduction.

Dissected human hair follicles were treated with the same concentrations of T3 andT4 as mentioned above, and also with TRH (10 ng/ml) and TSH (10 mU/ml). All four hormones showed promising effects on human hair follicles, as they increased MTCO1 and TFAM protein expression, stimulated the number of mitochondria in the hair follicle, as depicted by transmission electron microscopy, and again enhanced complex I activity.

These findings document complex stimulatory effects of T3 and T4 on mitochondrial function in human epidermis and of all HPT- members on hair follicle mitochondria, and identify these hormones as potent novel neuroendocrine stimulators of mitochondrial activity and biogenesis in human skin and hair follicles. Furthermore, we demonstrate that, contrary to conventional wisdom in mitochondrial research, human epidermis and dissected human hair follicles offer an excellent model system for dissecting neuroendocrine controls of human mitochondrial biology under physiologically relevant conditions.

P042
Expression of the IL-17 pathway in skin lesions of patients with hidradenitis suppurativa (acne inversa)

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Introduction: Hidradenitis suppurativa (acne inversa, HS) has been recently proposed to be classified in the 'autoinflammatory diseases', a group of recurrent, non-infectious inflammatory disorders, with typical absence of pathogens, autoantibodies or antigen-specific T cells. Interleukin (IL)-17, the signature cytokine of Th17 cells, has been proved to play a role in the pathogenesis of rheumatoid arthritis and Crohn's disease, disorders, which belong to 'auto inflammatory diseases' and present a high comorbidity with HS. Moreover, IL-17 is the founding member of a group of cytokines consisting the IL17-pathway and including IL-6, tumor necrosis factor (TNF)α, IL17-receptor, interferon-γ, IL4, IL13, IL23 and tumor growth factor (TGF)-β1. Other cytokines, which could be associated with the IL17-pathway, are TNFAIP3, a cytokine whose expression is rapidly induced by TNF, and FGL2, acytokine induced by IL4- and repressed by the TGF-β pathway. To corroborate the involvement of Th17 cell cytokines in HS we have investigated the IL-17 pathway in skin lesions of HS patients.

Objective: To investigate the expression of the IL-17 pathway in skin lesions of HS patients.

Methods: Gene array and immunohistochemistry experimemts were performed to detect the expression of cytokines involved to IL17-pathway in skin lesions of HS patients at RNA and protein levels. The results were compared to those of non-lesional skin from HS patients and of matched (same localization) skin specimens from healthy individuals. Moreover intestine specimens of patients with Crohn's disease were used as control.

Results: The gene array experiments detected a significantly higher IL17 expression in lesional skin compared to non-lesional skin of HS patients. Moreover, down-regulation of TNFAIP3 was found in non-lesional skin of female HS patients compared to healthy female individuals as well as lower expression of FGL2 in lesional skin compared to non-lesional skin of female HS patients. Immunohistochemical staining showed expression of FGL2 in the perivascular dermal tissue of lesional skin of HS patients, whereas fibroblasts, neutrophils and macrophages were stained. Positive FGL2 expression was also found in neutrophils, macrophages and dendritic cells in intestine sections of patients with Crohn's disease. IL17 expression was detected in neutrophils, lymphocytes, fibroblasts and mast cells in the dermis of HS lesions as well as in intestine specimens from patients with Crohn's disease.

Conclusion: Genes of the IL-17 pathway are expressed in inflammatory cells of HS lesions. It is likely that the IL-17 pathway is involved in HS pathogenesis like it is in Crohn's disease.

P043
Immunohistochemical detection of FoxO1 in human sebocytes

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The FoxO subfamily of transcription factors has a conserved role in the adaptation of cells and organisms to nutrient and growth factor availability. FoxO1 is a metabolic sensor for transcription and predominantly contributes to nutrigenomic regulation. Recently, a hypothesis of a relative nuclear deficiency of FoxO1 in the pathogenesis of acne has been proposed. Decreased nuclear levels of FoxO1 have been associated with increased androgen receptor transactivation, increased cell cycle progression, decreased apoptosis, increased lipogenesis, reduced oxidative stress resistance, increased T-helper cell proliferation and impaired innate immunity. All these biological functions correlate closely with the main steps in acne pathogenesis. Although it is known that FoxO1 is expressed in whole skin, no information of FoxO1 expression in the sebaceous gland was available. We performed an immunohistochemical study of six normal skin biopsies, rich in sebaceous glands, withanti-FoxO1 antibodies and could detect FoxO1 by the half of them in sebocytes and lymphocytes. Immunohistochemical staining of FoxO1 was strongest in nucleus. Our preliminary study shows that FoxO1 is expressed in human sebocytes and may thus be involved in sebocyte gene regulation and acne pathogenesis.

P044
The spleen as a conductor of neuro-immune regulation in allergic dermatitis -stress and Substance P dependent changes in neuro-immune communication

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The spleen is an important TH-2 inductor in healthy individuals preventing the body from an overwhelming inflammation i.e. after infection. It may however also play a role in TH-2 driven allergic disease. Splenic immune cells express a wide range of receptors for neuroendocrine mediators, and sympathetic activation as well as cortisol signalling usually drive the splenic TH-2 bias through an altered cytokine production of the responsive T-cells and antigen presenting cells in splenic white pulp. We here postulate that the main sensory neuropeptide and stress mediator Substance P (SP), which is known to promote a TH-1 bias and modify allergic inflammation in peripheral tissues, also acts as neuroendocrine immune-modulator in the spleen, possibly as a balancer in stress-dependent neuro-immune interaction and inflammatory skin diseases. To address this question, atopic dermatitis like allergic dermatitis (AlD) was induced in C57BL/6 mice by double sensitization (i.p) and an intradermal challenge using chicken egg ovalbumin. Animals were additionally exposed to sound stress for 24 h prior to challenge. Further the effect of various concentrations SP on APC and T-cells was determined in 24 h cell cultures. *In vivo* we found white pulp hyperinnervation 48 h after stress termination, increasing numbers of SP responding antigen presenting cells (APC) and increasing splenic expression of SP precursor PPT-1 and expression of NK-1 receptor. Nerve growth factor (NGF) – which is upstream of SP expression and neuronal plasticity -was also stress dependently up regulated. The TH-2 bias in AlD was reversed towards TH-1 mainly due to increased IFN-γ amounts. 72 h after stress termination NGF was no longer detectable but the expression of PPT-1 and NK-1 receptor was further increased and correlated to high TNF-α production. This was drastically diminished by blocking the SP receptor NK-1. *In vitro* low dose SP increased TH-1 characteristics on APC: IFN-γ and TNF-α were elevated and CD11c+/CD4+/CCR7+ and CD11c+/CD4+/MHC-II+ expression were increased. On T-cells TH-1 and regulatory/cytotoxic parameters were induced by high dose SP:down regulated IL-4 and elevated IFN-γ, strong increase of CD90+/CD28+ andCD90+/CD3+/CD8+ expression. Further analysis has to reveal whether dose dependent SP effects play a role in stress dependent control of splenic TH-2 bias and whether this plays a role in the development and progression of TH-2 dominated diseases such as AlD.

P045

Expression of Lympho-epithelial Kazal-type inhibitor (LEKTI)-2 in cutaneous squamous cell carcinoma

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Background: Recently, we discovered Lympho-epithelial Kazal-type inhibitor (LEKTI)-2 as a Kallikrein-related peptidase (KLK) 5 specific serine protease inhibitor expressed at palmoplantar sites of healthy individuals.

Objective: As protease inhibitors and proteases are important factors in the pathogenesis of tumorgenesis and often serve as markers for different carcinomas we asked whether LEKTI-2 in expressed in cutaneous squamous cell carcinoma (SCC).

Methods: Paraffin-embedded sections of SCC were stained by specific anti-LEKTI-2 antibodies using standards methods.

Results: LEKTI-2 immunoreactivity was detected at site of prominent hyperkeratosis of SCC. Immunostaining was detected in 11 out of 20 investigated tissue samples.

Conclusion: Our results show that LEKTI-2 expression is not limited to palmoplantar sites as reported previously. In abnormal differentiated keratinocytes of SCC, LEKTI-2 expression might lead to hyperkeratosis by inhibiting KLK5. As LEKTI-2 was not present in all tissue sections of SCC the benefit of LEKTI-2 antibodies as a diagnostic tool for detecting SCC is not recommended.

P046

Importance of macrophage migration inhibitory factor (MIF) in the pathogenesis of epithelial skin tumors

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Chronic exposure to solar ultraviolet irradiation stimulates the production of several cytokines in the skin which are known to be involved in the initiation of skin cancer. Recent studies have suggested a potentially role of macrophage migration inhibitory factor (MIF) in the UVB-induced pathogenesis of non-melanoma skin cancer including squamous cell carcinoma (SCC).

Our studies aimed to determine the pathophysiological function of MIF in epithelial non-melanoma skin tumors like SCCs and the effect of UVB irradiation on the MIF expression in normal human epidermal keratinocytes (NHEK), a keratinocyte cell line (HaCaT) and tumor cell lines (SCC12, SCC13).

To asses the role of MIF in the development and progression of non-melanomic skin cancer we performed immunohistochemical analysis of constitutive MIF expression in normal skin in comparison to actinic keratosis and squamous cell carcinoma. Our studies revealed a weak expression of MIF in normal skin which is constricted to individual keratinocytes in the basal layer. Skin samples of patients with actinic keratosis showed a predominant staining of nearly all keratinocytes in the basal and suprabasal layer. Furthermore we observed a very strong overexpression of MIF in tissue of SCC.

MIF can bind to the three receptor proteins CXCR2, CXCR4 and CD74. Immunofluorescence studies and FACS analysis of HaCaT cells have shown that an inflammatory stimulus like IFNγ is able to promote the upregulation of CD74.

To further elucidate the possible role of MIF in photocarcinogenesis, the UVB effect in the skin was examined by determination of the production of MIF induced in keratinocytes and tumor cells by UVB. ELISA studies showed a significant increase of MIF secretion in the supernatants of cultured NHEKs and HaCaTs 8 h after UVB irradiation with 120 mJ/cm^2, that increased in a time dependent manner up to 48 h. Furthermore an UVB-induced upregulation of the serum MIF content was seen inSCC12 and SCC13 cells, which also increased in a time dependent manner up to 48 h but was weaker in comparison to the upregulation of MIF of cultured keratinocytes and HaCaTs. Our data indicate that an increased production of MIF by UVB-treated skin cells may play an important role in UVB-induced pathogeneses of non-melanoma skin cancer.

P047

High levels of beta2-adrenoceptors in infantile capillary hemangiomas might mediate the strong therapeutic effect of propranolol

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Aims: Recently it has been shown that the non-selective beta-blocker propranolol strongly inhibits growth of infantile capillary hemangiomas and consecutively is proposed as therapeutic option in children suffering from disfiguring hemangiomas. The aim of this study was to evaluate our hypothesis that infantile capillary hemangiomas express high levels of beta 2-adrenoceptors which mediate the strong therapeutic effect of propranolol.

Methods: We performed immunohistochemistry analysis of 30 infantile hemangioma sections and 29 senile hemangiomas and healthy skin as controls. Staining for beta2-adrenoceptors was carried out on paraffin-embedded tissue sections. For quantification we used a scoring system which evaluates staining intensity and positively stained area.

Results: Both, infantile and senile hemangiomas express beta2-adrenoceptors. However, infantile hemangiomas showed a stronger staining for beta2-adrenoceptors, the difference in staining scores between infantile and senile hemangiomas was significant ($P < 0.0001$).

Conclusions: Infantile capillary hemangiomas express high levels of beta2-adrenoceptors which might mediate their strong responsiveness to the beta-blocker propranolol.

P048

Contact sensitization in the anal and genital area

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Background: A variety of cosmetic products, topical medicaments, and ointments and their ingredients as well as clothes and (moist) toilet paper come in contact to anogenital skin.

Objective: In order to give an update on the most frequent allergens causing allergic contact dermatitis in the anal and genital area, we analyzed the data of the Information Network of Departments of Dermatology (IVDK).

Methods: We analyzed the patch test results in 1374 patients suffering from dermatoses in the anogenital area ($n = 561$ genital dermatoses, $n = 470$ anal dermatoses, $n = 343$ anogenital dermatoses) patch tested in 44 dermatological departments of the Information Network of Departments of Dermatology (IVDK) from 2004 to 2008. All other patients patch tested during this time period without anogenital dermatoses formed the control group ($n = 49\,142$).

Results: Of the total study group, 662 (48.2%) patients were male. 179 (13%) had a past or present atopic dermatitis. The vast majority of the patients was older than 40 years of age ($n = 989$, 72%). Suspected allergen sources were first of all topical medicaments, followed by cosmetics, cleansing agents, clothes, rubber products, systemic medicaments and disinfectants. Allergic contact dermatitis was diagnosed in 409 (29.8%) of the tested patients. Patients with anogenital dermatoses were sensitized mainly to active agents of topical medicaments, in particular Bufexamac (5.3%). Sensitization pattern and sensitization rates observed in patients with genitaland anal involvement differed significantly. Patients with anal disease had significant higher sensitization rates for Bufexamac (9.4% vs 1.1%), fragrance mix I (8.7% vs 4.2%) and II (4.5% vs 2.6%), propolis (5.4% vs 1.9%), and MDBGN (6.3% vs 4.1%).

Conclusion: Patients with chronic anal dermatoses seem to have a higher risk to develop sensitizations to topically applied products and drugs than patients with genital dermatoses. Recommended patch test series (German Contact Dermatitis Research Group) are standard series, local anaesthetics series, topical antibiotics, antimycotics, steroids, ointment bases and preservative series as well as patients own products.

P049

Eczema in children and development of asthma and rhinitis: prospective longitudinal population-based Swedish cohort

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Background: The nature of the relationship between infant and pre-school eczema, and the later onset of asthma and rhinitis has been a matter of controversy. In children with eczema impaired skin barrier function may favour sensitization. Exposure of environmental and lifestyle factors might therefore contribute to the development of asthma and rhinitis in children with eczema.

Aim: To estimate the odds ratios (OR) of incident doctor diagnosed asthma and incident doctor diagnosed rhinitis in children with eczema in early childhood compared to children without eczema in early childhood. To investigate whether the relationship remains stable after adjustment for possible confounding variables.

Methods: The Dampness in Building and Health study started in the year 2000 in Värmland, Sweden. A parental questionnaire based on an ISAAC protocol was sent to all children aged 1–6 years. Five years later a follow-up questionnaire was sent to the children that were 1–3 years at baseline (response rate = 73%). Three thousand one hundred and twenty four children aged 1–2 participated in both surveys, 1556 were 1 year old and 1568 were 2 years old.

The prevalence of eczema at baseline was assessed; eczema was defined as 'eczema during the last 12 month'. Incident asthma was defined as having doctor diagnosed asthma in 2005 but not in 2000. Incident doctor diagnosed rhinitis was defined accordingly. The incidence rates for both asthma and rhinitis were 5-year cumulative incidence rates.

The unadjusted odds ratios of incident asthma and incident rhinitis were computed for children with eczema relative to children without eczema. In a multivariate logistic regression analysis adjusted odds ratios were computed by adjusting for a set of environmental and lifestyle factors.

Results: The prevalence of eczema during the last 12 month in 1–2 year old children was 551/3124 [17.6%, 95% confidence interval (CI) 16.3–19.0%]. The cumulative 5-year incidence of doctor diagnosed asthma was 150/2927 (5.1%, 95% CI 4.4–6.0) and of doctor diagnosed rhinitis 172/3080 (5.6%, 95% CI 4.8–6.5). Children aged 1–2 years with eczema at baseline had a more than twofold increased odds of developing doctor diagnosed asthma (crude OR 2.6, 95% CI 1.8–3.7) and more than three fold increased odds of developing doctor diagnosed rhinitis (crude OR 3.1, 95% CI 2.3–4.4) during the following 5 years compared to children without eczema. The odds of getting asthma and rhinitis remained significantly increased after adjustment forage, gender, onset of eczema, smoking of the parents, age at introduction of solid food, use of antibiotics, site of living and PVC material in home environment (adj. OR 2.7, 95% CI 1.9–3.8 and adj. OR 3.1, 95% CI 2.2–4.3, respectively).

Conclusion: Eczema in infancy and early childhood is strongly associated with incident asthma and rhinitis during the following 5-years. Early identification is therefore of great value for the prediction of the further course.

P050

State of the art of highly activated antiretroviral therapy (HAART) in Germany:current prescribing practices and regional differences

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Background and Objective: In Germany, 3000 cases of newly diagnosed HIV infections were estimated for 2009. This number raised constantly between 2001 and 2008 from 1443 to 2806, with a flattening since 2006. Treatment of HIV/AIDS improved distinctly within the last years. Not only many new and potent drugs of the well know substance classes protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI) and non-nucleoside reverse transcriptase inhibitors (NNRTI) were developed, which built the basis for the highly active antiretroviral therapy (HAART) since 1996. Also complete new substance classes were developed and mostly brought into standard medical practice. These act at different areas of the viral replication as integrase inhibitors (INI), and entry inhibitors (EI).

Knowledge on the prescribing practices of HAART in Germany is essential to evaluate the use of modern therapy approaches in clinical practice, the implementation of therapy guidelines and to foster health economical analyses. There is only very limited data on the prescribing practices of HAART in Germany and potential regional differences. On basis of the patient cohort of the Competence Network for HIV/AIDS (KompNet), we did a first analyses of related data.

Methods: The KompNet cohort is an open, retrospective and prospective, multi-center and nationwide cohort, representative for the German treatment situation regarding composition of sites and patients. Prospective documentation was started in 2004. It consist of 25 sites, covering epidemiological hotspots as well as more rural areas. Overall, it comprises approximately16 500 patients of which approximately 8300 are currently under follow up. The cohort covers 62 900 person years, 55 900 person years under HAART. With state of date 15.10.2009, we analysed mean CD4-cell count/μl at treatment initiation over time. Additionally, we evaluated prescription of single substances in 2009 as well as the current HAART regimens, both for Germany and comparing the sites of Bochum/Essen (BO/E) and all other sites (REST).

Results: Mean CD4-cell-count/μl at treatment initiation raised constantly from 289 in 2001 to 349 in 2009. In 2009, a total of 10 044 prescribed substances (BO: 1866) and a total of 4577 HAART-regimen (BO/E: 948) were documented. Regarding the whole cohort, nearly all regimen contained NRTI (95.5%), followed by PI (52.2%), NNRTI (42.9%), INI (6.9%) and EI (2.4%). 8.6% were class-saving regimen, 5.8% solely based on NRTI, 2.8% on PI. Regarding prescription practice of single substances, Truvada had the highest proportion of all by far (18.6%), followed by Kaletra (9.6%), and Viramune (9.5%). Innovative drugs were prescribed more seldom (Isentress: 3.0%, Celsentri: 0.7%). As to combined regimen, the latter were used more frequent having more regimen changed (≥3 changes: Isentress: 8.1% of allregimen, Celsentri: 1.8%). Partly, we found differences in prescription practices between BO/E and REST, especially concerning Kaletra (BO/E: 11.7%; REST: 9.1%), Combivir (BO/E: 9.2%; REST: 5.6%) and Atripla (BO/E: 7.0%; REST: 3.7%).

Discussion: The constant increase of CD4-cell-count/μl at start of HAART reflects the movement to an earlier treatment initiatio.

P051
Outpatient care of atopic eczema: Secondary data analysis indicates a high degree of heterogeneity in prescribing patterns of medical providers

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Background: An important but yet unknown issue in health services research concerns the degree of variation and the reasons for variation in the treatment of atopic eczema (AE) by medical providers in outpatient care.
Methods: Secondary data analysis utilizing an administrative health care database from Germany with complete information on outpatient health services utilisation and prescription data of 257.347 individuals, 11.555 of which (4.5%) were classified as having prevalent AE. Within the 2-year observation period (2003/4), a total of 71 dermatologists and 175 paediatricians provided medical services for patients with AE. We assessed and compared the proportions of patients with AE that were prescribed topical corticosteroids, topical tacrolimus, and topical pimecrolimus by individual dermatologists and paediatricians. We analyzed the variation in prescribing patterns within and between medical disciplines considering age, gender, and the total number of patients with AE treated by an individual physician as potential explanatory factors for heterogeneity.
Results: The mean (fifth to 95th percentile) proportion of AE-patients treated by dermatologists and paediatricians were 160 (9–379) and 31 (3–91), respectively. The median proportion of patients prescribed topical corticosteroids by dermatologists and paediatricians was 43% and 35%, respectively. Paediatricians were more likely to prescribe topical pimecrolimus (median proportion of patients treated by dermatologists versus paediatricians: 21% vs 5%). There was a pronounced degree of heterogeneity of anti-inflammatory treatment among dermatologists and paediatricians which was neither explained by the age or sex of patients, nor by the total number of AE-patients treated by the individual provider. The mean (fifth to 95th percentile) proportion of patients prescribed potent topical corticosteroids and topical tacrolimus by dermatologists treating more than 200 patients with AE within the 2-year observation period ($n = 17$) was 31% (3–51%) and 1% (0–5%), respectively.
Conclusion: There is an unexpected high degree of heterogeneity in prescribing patterns of topical anti-eczematous treatments among dermatologists as well as paediatricians. Neither patients' demographics nor the absolute number of AE-patients as a surrogate parameter of experience and specialization of the provider explain the observed variation in prescribing patterns. Indirectly, this study indicates a high potential to improve outpatient care of AE through better standardization of treatment strategies. Future qualitative and quantitative research is needed to better understand the reasons for variations in prescribing patterns and to eventually inform targeted health services research interventions.

P052
Update of epidemiologic and clinical data of Adamantiades-Behet's Disease in Germany (2010)

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The German Registry of Adamantiades-Behet's Disease (ABD) is a registered charity, founded 1990 and providing epidemiologic data for ABD in permanent residents of Germany. The current prevalence of ABD based on this data is 0.88:100 000. Of the 421 male and 304 female ABD patients, 287 are of German origin (39.6%), 317 of Turkish origin (43.7%), and 12 of Italian (1.7%), 10 of Greek (1.4%), and nine of Lebanese origin (1.2%). Another 83 patients originate from 27 other countries. The frequencies of major clinical manifestations are: oral ulcers 98.5%, skin lesions 74.4%, genital ulcers 63.8%, arthritis 52.1%, ocular manifestations 51.6%, and pos. pathergy test 30.5%. Severe ocular involvement is significantly associated with HLA-B5 ($P < 0.001$) and male gender ($P = 0.001$). Oral ulcers were with 83.8% the most common onset, genital ulcers with 40.9% the most frequent second sign. Among skin lesions, papulopustules could be detected in 58.5%, erythema nodosum-type lesions in 37.5%, pyoderma in 11.8%, thrombophlebitis in 11.3%, and skin ulcers in 10.8%. Verified CNS involvement was diagnosed in 11.8%, gastrointestinal involvement in 11.1%, prostatitis/ epididymitis in 10.5%, lung and cardiac manifestation in 3.5% and 2.7%, resp., renal vasculitis in 1.8%. Severe courses occurred in 10.9%, fatal outcome in 1.2%, $n = 7$. Median age of onset is 28 years (range 0–72 years). The complete clinical picture developed in 5 months (median). Interval between onset and diagnosis is 50 months (median) being significantly longer than the duration of development of the complete clinical picture ($P < 0.001$).

P053
Differences in the distribution of clinical signs between Adamantiades-Behet's Disease patients of Turkish and German origin in Germany

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Among the 725 patients with Adamantiades-Behet's Disease reported to the German Registry of Adamantiades-Behet's Disease until 2010, 317 were of Turkish (43.7%) and 287 of German origin (39.6%). Androtopism was found among patients of Turkish origin (men:women = 1.84:1, $P < 0.001$), while no gender predilection was recorded in German patients (men:women = 0.93:1, ns). Altogether 9.3% of patients showed a positive family history with significant difference among Turkish and German patients (14.3% vs 3.7%, respectively, $P < 0.001$). In general, there was a trend of more frequent ocular involvement in Turkish than in German patients (59.6% vs 49.7%, $P = 0.044$), but there was no statistical difference in the risk of blindness (8.7% vs 4.5%; $n = 18$ vs 8). A similar trend could be observed for folliculitis being more frequent in Turks than in Germans (67.9% vs 58.7%, $P = 0.05$), whereas sterile prostatitis/epidimytitis was more frequent in German patients (17.4% vs 7.6%, $P = 0.02$).48.3% of the patients had HLA-B5 antigen; of these 64.5% were of Turkish and 35.5% of German origin ($P < 0.001$). Frequencies of other clinical signs showed no significant differences between Turks and Germans: oral aphthae (98.9% vs 98.0%), genital ulcers (63.2% vs 67.1%), arthralgia (50.2% vs 51.3%), erythema nodosum (42.5% vs 35.1%), thrombophlebitis (13.1% vs 13.4%), gastrointestinal (10.1% vs 15.2%) and CNS involvement (9.7% vs 12.0%), pos. pathergy test (31.5% vs 30.5%). In the Turkish as well as in the German patients group, oral aphthae were the most common first (85.6% vs 84.7%, ns), genital ulcers the most common second symptom (52.7% vs 47.3%, ns).

P054
Significant association between atopic eczema and attention-deficit/ hyperactivity disorder – pooled results of four population-based studies

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Background: Recently we performed four large population-based epidemiologicalstudies consistently indicating an association between atopic eczema (AE) and attention-deficit/hyperactivity disorder (ADHD) independent from sociodemographic factors, environmental confounders, and comorbidities. Although the epidemiologic evidence is consistent and suggests that AE precedes the onset of ADHD, the absolute importance of the newly described comorbidity between AE and ADHD on the population level and its relative importance compared to the established comorbidities between AE and allergic rhinitis/asthma deserves further investigation.
Methods: We performed a random-effects meta-analysis of the four recently published epidemiological studies concerning the association of AE and ADHD/ADHD-symptoms and compared the pooled odds ratio (OR) for the association between AE and ADHD with the pooled ORs for the association between AE and allergic rhinitis and between AE and asthma. ORs from the individual studies were adjusted for age, sex, and atopic comorbidities. We also calculated the population attributable risk (PAR) for each comorbidity assuming a lifetime prevalence of AE of 25%.
Results: The pooled OR [95%-confidence interval (95% CI)] for the association of AE and ADHD, allergic rhinitis and asthma were 1.466 (1.284–1.674), 2.716 (2.297–3.211), and 2.390 (2.089–2.735), respectively. The pooled PAR (95% CI) of ADHD, allergic rhinitis, and asthma presumably attributable to AE were 10.0% (6.6–14.4%), 25.8% (21.3–30.3%), and 30.0% (24.5–35.5%), respectively.
Conclusions: Children with AE have an approximately 50% increased risk to be diagnosed as having ADHD compared to children without current or previous AE. The established association between AE and other atopic conditions is about twice as strong as the newly observed association between AE and ADHD. Assuming a causal relationship between AE and ADHD 10% of all ADHD cases are potentially preventable through targeted primary prevention measures in children with AE. Therefore, experimental studies focussing on the underlying biological mechanisms related to AE that may cause ADHD-symptoms are of high public health relevance.

P055
Potential psychoneuroimmunological mechanisms to explain the comorbidity between atopic eczema and attention deficit/hyperactivity disorder

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Epidemiological data suggest that atopic eczema (AE) in infancy significantly increases the risk for attention deficit/hyperactivity disorder (ADHD) in later life. The underlying pathophysiological mechanisms of a link between AE and ADHD are unknown. We propose two potential, not mutually exclusive models that may explain why AE and ADHD are correlated. Accumulating data indicate the impact of pro-and anti-inflammatory cytokines as well as early life stress on prefrontal cortex regions and neurotransmitter systems such as the dopaminergic and the serotonergic system known to be involved in executive cognitive functions and attention. We propose that in the AE child, a sustained and exaggerated release of cytokines during the chronicallergic inflammatory process and/or elevated levels of psychological stress as a result of the chronic disease interfere with the maturation of these specific brain regions and neurotransmitter systems leading to an increased risk for ADHD. Alternatively or additionally, ADHD and AE may share one or several (yet unknown) overlapping risk factors that increase the susceptibility to both disorders leading to the co-occurrence of AE and ADHD. Genetics and prenatal stress exposure have been recognized to be relevant factors in AE and ADHD pathology and are discussed to be two candidates to contribute to co-occurrence of AE and ADHD. Future research, however, is needed to further explore and evaluate these models which may lead to a better understanding how psychosocial factors might interact with neurobiological and genetic factors to impact AE and ADHD.

P056
LEOS – lymphedema outcomes study: quality of lymphedema care in Hamburg

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Introduction: Today, lip- and lymphedema can be effectively treated with compression and manual lymph drainage. However, clinical experience shows that many patients do not receive adequate diagnosis and therapy.
Methods: LEOS is a non-interventional, cross-sectional quality of care study. Patients with lip and lymphedema were recruited via medical stores, physicians, lymph therapists and newspapers. They were interviewed on their former and current edema care, concomitant diseases, and costs of illness. With patient questionnaires, data on quality of life, treatment benefit, and satisfaction with care were collected.
Results: Data of $n = 348$ patients could be analyzed; $n = 301$ also sent back the patient questionnaires. 91% were female. 65% had a leg edema, 18% an arm edema, and 18% had both. Seventy one percent were currently treated in the 'Hamburger Lymphnetz', a network of lymph specialists. 17% had consulted a physician only 5 or more years after the first symptom. In 22%, 5 or more years had passed between the first physician consultation and diagnosis of the edema. 86% currently received lymph drainage, 87% compression therapy.
Most frequent concomitant diseases were adipositas (48%), arterial hypertension (44%) and chronic venous insufficiency (31%). Sixty six percent rated their quality of care as good/very good, 6% as quite bad/bad. Among those 49 patients who had been recruited via newspapers, only 41% rated their quality of care as good/very good and 21% as quite bad/bad. Beside the reduction of swelling, the most important treatment goal was to receive optimal compression stockings (quite or very important to 84% of the patients).
Discussion: The patients taking part in this study received rather good edema care at the time of the interview. Nevertheless, the high percentage of patients who reported that they had visited a physician and received the correct diagnosis only many years after the first symptom and the markedly lower satisfaction with care in patients recruited via newspapers indicates that there are significant deficiencies in lymphedema care.
Conclusion: To prevent misdiagnoses of lip- and lymphedemas and to thereby set the stage for correct treatment, better information of both patients and health care providers is necessary.

P057
Effect of age on patient preferences for psoriasis treatment processes and outcomes

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Background: Psoriasis is a common, chronic disease often requiring lifelong treatment. Greater satisfaction and adherence with treatment have been reported when treatment recommendations fit well with patients' characteristics (e.g. gender, age, sex, etc.) and preferences for treatment attributes. Recognizing patient preferences in treatment decision-making, therefore, may be an efficient way of finding the most effective, patient-acceptable treatment.
Objectives: Our study explored the effect of patients' age on psoriasis patients' preferences for hypothetical treatment scenarios.
Methods: Participants included patients identified as having moderate to severe psoriasis according to the criteria of the Committee for Medical Product for Human Use attending weekly outpatient psoriasis clinics in the Department of Dermatology at the University Medical Centre Mannheim, Heidelberg University. Both new patients and patients who previously attended the outpatient clinics were eligible for the study. Participants with psoriatic arthritis, but no skin involvement, and patients <18 years of age were excluded. Conjoint analysis was utilized to measure participants' stated preferences for attributes of psoriasis treatment options. Available psoriasis treatment modalities were identified through literature review and consultations with clinical experts. The treatments modalities were decomposed into attributes and attribute levels. Treatment attributes included both process (treatment location, frequency, duration, delivery method, and cost for the individual) and outcome (probability of beneficial effect, magnitude of beneficial effect, duration of benefit, probability of side effects, side effect severity, and side effect reversibility) attributes. Using Sawtooth Software (www.sawtoothsoftware.com), hypothetical treatments cenarios were created. Participants were asked to repetitively choose their preferred treatment option among the pairs of hypothetical treatment scenarios presented. Multivariate regression analysis was performed to investigate the effect of participant characteristics, including age, on the relative importance (partworth utilities) measured for each treatment attribute.
Results: The study sample (n = 163, 58.9% male) included 17% between the ages of 18 and 35 years, 34% between the ages of 36 and 49 years, 32% between the ages of 50 and 64 years, and 17% >65 years old (mean age 49.27 years). The mean PASI was 5.58 and mean DLQI was 7.58. Patients' preferences for treatment attributes were found to vary among the age groups analyzed. Younger patients valued the possibility of beneficial effect (and outcome attribute) more than older patients (P = 0.003), while older patients valued the method of treatment delivery (a process attribute) more than their younger counterparts (P = 0.02).
Conclusions: Psoriasis patients of different age appear to have significantly different treatment preferences. Opportunities to improve treatment adherence, clinical outcomes, and satisfaction with care may be overlooked if the influence of patient characteristics, such as the age of the patient, on treatment preferences are overlooked.

P058
Socioeconomic factors may influence patients' access of psoriasis treatments

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Background: Rates of treatment dissatisfaction and non-adherence are high among psoriasis patients, in part due to discordance between treatment modalities and individuals' treatment preferences, social and work contexts.
Objectives: The purpose of our study was to assess the influence of socioeconomic factors on patients' preferences for process and outcome attributes of psoriasis treatments.
Methods: Participants with moderate to severe psoriasis according to the criteria of the Committee for Medicinal Products for Human Use four attending outpatient dermatology clinics at the University Hospital of Mannheim, Germany, were recruited, including new patients and those with ongoing therapy. Currently available treatments were decomposed into general process (treatment location, frequency, duration, delivery method, and cost for the individual) and outcome (probability of beneficial effect, magnitude of beneficial effect, duration of benefit, possibility of side effects, side effect severity, and side effect reversibility) attributes and attribute levels. Theoretical treatment options based on random combinations of the identified attributes and levels were created using commercially available software (www.sawtoothsoftware.com). Participants were asked to choose the preferred alternative among pairs of options. Relative attribute importance of each attribute was calculated to asses the influence of treatment attributes on participants' choices. Multivariate regression analysis was performed to analyze the impact of socioeconomic characteristics on relative attribute importance.
Results: Participants (n = 163, 58.9% males, average age 49.27 years) demonstrated a mean PASI of 5.58 and DLQI of 7.58. For the sample, the most important attribute was treatment location [relative importance score (RIS) = 26.76], followed by possibility of beneficial effect (RIS = 23.77) and delivery method (RIS = 23.49).Participants with a monthly household income between 1000 and 2000 EUR were more concerned about the duration of beneficial effect (P = 0.024) and treatment frequency (P = 0.078), with severity of side effects (P = 0.027) and delivery method (P = 0.005) less important, as compared to participants in other income categories. The importance of cost of treatment did not vary significantly between income groups.
Conclusion: Income does not appear to influence patients' willingness to pay for psoriasis treatments, but does appear to influence their preferences for treatment attributes. The observed differences in treatment preferences may reflect the influence of work status and structure on the ability of patients to access care.

P059
Comorbidity in psoriasis vulgaris: an overlooked opportunity to improve delivery of care

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Background: Psoriasis is a common chronic inflammatory disease with profound effects on individuals' quality of life. A broad range of treatment options are available. However, rates of non-adherence are high, partly due to discordance between treatment modalities and patients' preferences.
Objectives: We investigated the influence of comorbidities on patients' treatment preferences.
Methods: Participants with moderate to severe psoriasis (according to the Committee for Medical Products for Human Use) attending outpatient dermatology clinics at the University Hospital of Mannheim, Germany, completed an online survey including conjoint analysis exercises. Currently available treatments were decomposed into process (treatment location, frequency, duration, delivery method, and cost for the individual) and outcome (probability of beneficial effect, magnitude of beneficial effect, duration of benefit, probability of side effects, side effect severity, and side effect reversibility) attributes and attribute levels. Hypothetical treatment options were generated using commercially available software (www.sawtoothsoftware.com) and participants were asked to choose preferred scenarios from a series of scenario pairs. Multiple regression analysis was performed to analyze the influence of comorbidity on the relative importance (partworth utilities) measured for each treatment attribute.
Results: Participants (n = 163, 58.9% males, average age 49.27 years) had a mean PASI of 5.58 and DLQI of 7.58. Twenty-seven percent were diagnosed with psoriatic arthritis, 13.5% reported cardiovascular disorders, 12.9% reported depression and 8% reported diabetes mellitus. When evaluating aggregate preferences, treatment location was considered the most important attribute, followed by possibility of plaque reduction and delivery method. In subgroup analysis, preferences varied significantly depending on comorbidities present. For example, participants with psoriatic arthritis cared more about the possibility of beneficial effect achieved by treatment (P = 0.037) and participants with cardiovascular disorders put more weight on the possibility of side effects (P = 0.046) as compared to those without these conditions. Perhaps most note worthy, patients with depression attached less importance to the magnitude of beneficial effects (P = 0.017), but more importance to treatment duration (P = 0.023).
Conclusion: Our results highlight the influence of comorbidity on patients' treatment preferences. Consequently, outcomes may be improved if the interaction between patients' comorbidities and treatment preferences is considered in treatment recommendations, facilitating improved adherence. Specifically, addressing patients' depression may be a currently overlooked opportunity to improve delivery of care.

P060
Is attractiveness more important than a healthy skin? Results from a first population-based pilot study on characteristics and motives of sunbed users in Germany

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Introduction: Skin cancer incidence increases dramatically in Germany and other nations with about 80–90% of cases attributable to ultraviolet (UV) radiation. Besides sunlight, the use of UV-emitting tanning devices is one of the most important risk factors for skin cancer and was therefore classified as carcinogenic by the International Agency for Research on Cancer (IARC) in 2009. However, while millions of people worldwide deliberately expose themselves to unnecessary UV radiation by sunbeds, little is known about the motives and characteristics of sunbed users.
Methods: The SUN-Study 2008 ('Sunbed-Use: Needs for Action-Study 2008'), a pilot study of an ongoing nationwide survey, is the first population-based study on this topic conducted by the Mannheim Institute of Public Health (MIPH) in collaboration with the Association of Dermatological Prevention (ADP). Five-hundred persons aged 18–45 living in Mannheim were asked in telephone interviews about sunbed use, motives, skin type and lifestyle. Besides descriptive analyses we conducted chi-square tests to investigate if those using sunbeds to enhance attractiveness differ from other users.
Results: Every second respondent had used a sunbed at least once (46.7%; ever users), whereas one fifth had used it during the last year (21.0%; current users). The majority of ever users knew that sunbed use can have serious consequences for health. Enhancing attractiveness was the most important motive for sunbed use indicated by 62.0% of 234 ever users. Compared to others, those using sunbeds to enhance attractiveness had more often skin type III/IV (68.6% vs 50.0%, P = 0.005), used sunbeds predominantly in tanning salons (67.5% vs 29.4%, P < 0.001) and had already had sunburn after sunbed use (71.1% vs 53.9%, P = 0.005). Furthermore, enhancing attractiveness was even more important for current users than for past users (78.1% vs 48.8%, P < 0.001), who rather used sunbeds for pretanning and other reasons.
Discussion: Although most users are aware of the risk of sunbed use, they nonetheless use tanning devices for superficial reasons such as enhancing attractiveness. The association between using sunbeds to enhance attractiveness and past sunburns after sunbed use emphasizes the risk-taking characteristic of sunbed users. This implies that classical education campaigns will hardly be successful in preventing sunbed use in the bigger part of users who share this motive. Instead, change in the public opinion is needed including that an immoderate tanned skin should not longer be aesthetically admirable.

P061
Epidemiological and immunological investigations on cutaneous leishmaniasis (CL) and Leishmania/HIV co-infection in Cameroon

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Approximately 350million people are at risk of acquiring leishmaniasis worldwide. The spread of HIV expanded the endemicity of leishmaniasis significantly, since it is an opportunistic infection in HIV-infected individuals. We have conducted an epidemiological and immunological study on CL and CL/HIV co-infection in Northern Cameroon. Such studies are of great public health importance as both diseases occur in the region and successful control programs against HIV should integrate opportunistic infections such as leishmaniasis. Of 32 466 persons screened, 146 presented active CL lesions (0.5%) induced by L. major and an additional 261 (0.8%) had scars indicative of previous CL infection (past cases). Clinically, the disease ranged from localized to disseminated CL with the number of lesions varying from 1 to 19 per individual. HIV serological testing identified seven (4.8%) HIV+ patients (five HIV-1, two HIV-1/2). Several clinical parameters such as the numbers of CL lesions and lesion sizes were larger and the time to lesion resolution was longer in HIV co-infected individuals as compared to HIV negative controls. Next, we characterized the underlying cellular and humoral immune mechanisms for susceptibility to Leishmania and HIV. In serum, we detected elevated levels of Leishmania-specific IgG in all samples; however, significantly lower levels were found in HIV co-infected subjects. Isotype-specific differences were not obvious. In addition, multiplex analysis of Th1/Th2 cytokines revealed significantly decreased levels of IL-6 and IL-8 in samples of HIV co-infected patients, but higher amounts of the Th2-associatedcytokines IL-4 and IL-5. Analyses of skin biopsies obtained at different time points showed fewer epidermal LC, CD1a+ dermal DC, CD68+ macrophages, as well as fewer CD4+ T cells and CD20+ B cells in HIV co-infected individuals. In summary, we demonstrated Leishmania/HIVco-infections in Cameroon in approximately 1/20 CL patients. Also, our results confirm prior studies demonstrating worsened disease outcome in Leishmania/HIV co-infected as compared to HIV negative patients indicating that an increased susceptibility to progressive disease after infection with this otherwise dermatotropic strain (L. major) is observed in the HIV+ patients. Finally, our immunological studies suggest severe alterations in the protective immune response initiated by antigen presenting cells and mediated by IFN gamma-producing T cells. A detailed understanding of the immunological responses in Leishmania/HIVco-infected individuals may aid the development of optimized therapeutic regimens for this severely affected group.

P062
Relevance of the psoriasis area and severity index (PASI) regarding patient-defined benefit in the therapy of psoriasis

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Background: The 'Psoriasis Area and Severity Index' (PASI) is used to document the course of disease. Improvements in the PASI score of 50% resp. 75% (PASI-50 PASI-75) have been established as relevant cut points. Besides such objective clinical outcomes, patient-defined benefit becomes increasingly important in evaluation of therapies in many European countries.
Objective: To analyze to what extent the clinical parameters PASI-50 and PASI-75 reflect the individually defined patient benefit.
Methods: A prospective observational study of outpatients with psoriasis vulgaris was conducted. Data, obtained by questionnaires at two points in time (t1, t2) during the study period, encompassed PASI scores, therapy of psoriasis and (only t2) global questions on satisfaction with therapy and medical care. As a measure of patient-defined outcomes the Patient Benefit Index (PBI) was assessed, an instrument developed and validated in Dermatology: Prior to therapy (t1) patient's needs and after therapy (t2) the degree to which these were achieved are obtained, resulting in the calculation of an individually weighted single outcome parameter (PBI range 0–4; PBI ≥ 1 is considered as minimum relevant benefit).
Results: Data of 93 patients were suitable for analysis. Mean PASI was 13.7 (9.5) att1 and 5.5 (5.9) at t2, which equals an improvement about 60%. 72.5% of the patients reached PASI-50 and 32.5% PASI-75. Mean PBI was 2.3 (1.3), 76.3% showed a PBI of ≥1.
Improvements in the PASI-Score were significantly correlated with the PBI ($R = 0.45$; $P \leq 0.01$) however, associations of both parameters to global items of patient satisfaction differed substantially: From the patients whose expectations have been met completety, 100% had a PBI ≥ 1, 89.0% PASI-50 and only 55.6% PASI-75. By contrast, from the patients, whose expectations have not been met at all, none had a PBI ≥ 1 or PASI-75 but 50.0% had PASI-50. Equally, in the group of patients who stated they would undergo the same therapy again, the proportion of PBI ≥ 1 was 81.3%, of PASI-50 72% and of PASI-75 only 35%. From the patients who answered, they would never do the same therapy there was no patient with PBI ≥ 1 or with PASI-75 but 20% of this very unsatisfied group had PASI-50.
Conclusions: Even though change in PASI is associated with the patient-defined benefit of therapy it does only reflect it incompletely. Whereas the full range of therapy evaluation is reproduced well by the PBI, PASI-50 corresponds mainly to the upper and PASI-75 to the lower ranges of satisfaction. I.e. there are a substantial number of unsatisfied patients who still have PASI-50 and an even greater number of patients who did not reach PASI-75 but experienced a remarkable individual benefit from the therapy.
In addition to the PASI, specific assessment of patient-defined benefit should be implemented in evaluation of psoriasis therapy.

P063
Overweight, obesity and atopic diseases: evidence from a population-based cross-sectional study in Germany (KiGGS)

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The purpose of this study was to analyse the association of overweight and obesity with hay fever, eczema and asthma in German children and adolescents.
Data was drawn from the public use files of the German Interview and Examination Survey for Children and Adolescents (KIGGS), a nationwide cross-sectional representative survey conducted between 2003 and 2006. The association of hay fever, eczema and asthma with overweight and obesity was analysed by means of multivariable logistic regression, using proc surveylogistic in SAS. Age, social position, migrant status, East/West, living area, less than one older sibling, ever fully breastfed, maternal smoking during pregnancy, dog, small furry pet, infection afterbirth, underweight/pre-term delivery, parental allergies, maternal BMI, paternal BMI and physical activity were adjusted for. Overweight and obesity were defined using German reference values (>90th and >97th age and sex-specific percentile, respectively) according to Kromeyer-Hauschild *et al.*
Multivariable analyses revealed a significant positive association of ever physician-diagnosed asthma with overweight in boys (OR = 1.71, 95% CI 1.22–2.40, $N = 4366$), but not in girls (OR = 1.26, 95% CI 0.79–2.03, $N = 4063$). Everphysician-diagnosed eczema was unrelated to overweight in girls (OR = 1.08, 95% CI 0.80–1.44, $N = 4063$) and boys (OR = 0.97, 95% CI 0.72–1.30, $N = 4366$). The same was true for ever-physician diagnosed hayfever (girls: OR = 1.03, 95% CI 0.70–1.51, $N = 4063$; boys: OR = 1.13, 95% CI 0.84–1.53, $N = 4366$). Allergic sensitization was significantly associated with overweight in boys (OR = 1.33, 95% CI 1.05–1.68, $N = 3874$), but not girls (OR = 1.01, 95% CI 0.79–1.30, $N = 3564$).
A different pattern emerged for obesity. There was a significant positive association of asthma with obesity in girls (OR = 1.89, 95% CI 1.04–3.44, $N = 3736$), but not in boys (OR = 1.58, 95% CI 0.95–2.65, $N = 4013$). Eczema was unrelated to obesity in both girls (OR = 1.05, 95% CI 0.67–1.65, $N = 3736$) and boys (OR = 0.87, 95% CI 0.56–1.34, $N = 4013$) and so was hayfever (girls: OR = 1.57, 95% CI 0.91–2.69, $N = 3736$; boys: OR = 1.19, 95% CI 0.74–1.91, $N = 4013$) as well as allergic sensitization (girls: OR = 1.17, 95% CI 0.81–1.69, $N = 3292$; boys: OR = 1.18, 95% CI 0.84–1.67, $N = 3553$). Asthma, but not hayfever or eczema, is significantly associated with overweight in boys and with obesity in girls. Allergic sensitization seems to be related to overweight in boys only. These findings call for more research investigating the differential development of allergy/atopy and overweight/obesity over the life course, particularly in childhood and adolescence.

P064
Is the impact of atopic disease on children and adolescents' health related quality of life modified by mental health? Results from a population-basedcross-sectional study

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Eczema (previously termed atopic eczema or atopic dermatitis), hay fever and asthma are global health problems and their prevalence has increased considerably over the last decades. All appear to share an underlying atopic diathesis but their aetiology is considered to be multifactorial. The three conditions have been linked to decreases in health related quality of life (HRQoL) in adults, children/adolescents or parents of children. Research also suggests an association of the three conditions with mental health, which in turn is related to HRQoL decreases.
The impact of occurrence of the three conditions within the past 4 weeks on HRQoL was analysed by use of the complex sample general linear model in a population-based sample ($N = 6518$) of children and adolescents aged 11–17. Analyses were adjusted for the other atopic conditions, sociodemographic and clinical variables and stratified for mental health as measured by the Strengths and Difficulties Questionnaire (normal $n = 5697$, borderline $n = 609$, abnormal $n = 193$).
Eczema (B = -1.82, $P = 0.015$) and hay fever (B = -1.46, $P = 0.020$) within the past 4 weeks were significantly associated with decreased HRQoL after adjusting for the all other variables when no mental health abnormalities were present. No significant associations between these two atopic condition and HRQoL were observed in the groups with borderline or abnormal mental health, respectively. No impact of asthma within the past 4 weeks in any of the groups was observed.
While the results suggest mental health to have a modifying effect on the relationship between some atopic conditions and HRQoL caution needs to be exercised in interpreting the results as the groups with borderline or abnormal mental health were comparably smaller than the group with normal mental health. In the group with normal mental health small effects were more likely to become significant than in the other two groups.

P065
A novel homozygous missense mutation in SLURP1 causes Mal de Meleda with an atypical phenotype

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Mutations in SLURP1, encoding the secretedmammalian-Ly6/urokinase-plasminogen-receptor-related-protein-1 (SLURP-1), result in Mal de Meleda (MDM), a rare autosomal recessive form of keratoderma mainly characterized by sharply demarcated palmoplantar erythema and hyperkeratoses with progressive transgression to the dorsal aspects of the hands and feet. Phenotype variation has been reported to vary with geographic origin and ethnic background of the patients.
A 27-year-old Austrian of Turkish origin presented with an atypical phenotype of MDM without involvement of the plantar skin and missing of other previously reported associated clinical findings. Family history did not reveal any affected relatives, and there was no known consanguinity. Histology was consistent with MDM. Transmission electron microscopy demonstrated normal intermediate filaments and corneodesmosomes, and non-specific irregularly shaped keratohyalin granules with a spongy appearance. Genetic analysis revealed a novel homozygous missense mutation in exon 3 of SLURP1, namely p.Pro82Ser (*c*.244C > T). The mutation was not found on 100 chromosomes of unaffected controls. Altering a proline residue to serine would be predicted to cause a significant change in protein structure. Treatment consisted of oral acitretin 20 mg/day and topical antimicrobial and keratolytic therapy, which markedly improved the patients skin condition.
The present case expands the spectrum of clinical phenotypes associated with mutations in SLURP1 and provides new information on the allelic heterogeneity of MDM.

P066
Loss of corneodesmosin leads to peeling skin disease: mutation spectrum expanded

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Generalized inflammatory peeling skin disease (PSD) (MIM 270300) is an unusual autosomal recessive congenital ichthyosiform erythroderma, which is characterized by lifelong patchy peeling of the entire skin, pruritus and associated atopic diseases.
We recently characterized a large consanguineous family with PSD and carried out whole-genome linkage analysis, which identified a candidate region on chromosome 6p21.3, and for the first time identified recessive early N-terminal nonsense mutations in CDSN, the gene encoding corneodesmosin. Corneodesmosin is an important adhesive protein of the extracellular part of the corneodesmosomes and vastly important for the epidermal barrier integrity.
In another family we now established the diagnosis of PSD and in the patient confirmed the compound heterozygous CDSN genotype p.L59X/p.P344PfcX121. The second nonsense mutation represents a deletion (*c*.1031delC) with consecutive frameshift and premature stop codon and predicts truncation of the protein near the N-terminal start of the second glycine loop of corneodesmosin.
The results represent the first confirmation that PSD is due to recessive nonsense mutations in CDSN. Interestingly, the novel mutation p. P344PfxX121 is located C-terminal of the functionally important first glycine loop, hence suggesting a greater spectrum of CDSN mutations in peeling skin disease.

P067
A new 3′ trans-splicing repair strategy for the COL17A1 gene

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Mutations on both alleles of the collagen 17 gene (COL17A1) are the cause fornon-Herlitz junctional epidermolysis bullosa (nH-EBJ). Patients affected by this disease show generalized blistering of skin and mucous membranes in combination with nail dystrophy. Besides symptomatic wound care a gene repair approach is away of causative therapy. Trans-splicing is a technology that can reprogram a gene of interest on the RNA level. Instead of delivering a full-length transgene, trans-splicing repair requires only the part of the gene to be replaced. We demonstrated previousely, that spliceosome mediated RNA trans-splicing is applicable for the COL17A1 gene by targeting intron 51 with a 3′ pre-trans-splicing molecule (PTM). This PTM consists of a binding domain (BD) specific for intron 51, splice elements to perform the trans-splicing reaction and the 3′ exons of COL17A1.To be able to treat all EBJ patients with just two molecules (5′ and 3′ repair) we decided to target another intron. Thus the COL17A1 sequence was analysed to identify an intron in the middle of the gene in order to repair mutations over a region of 2000 nucleotides. In previous studies we have already demonstrated that the binding domain is crucial for efficency of the trans-splicing reaction. Hence a fluorecent based system to screen for optimal binding domains was established and adapted for the selected intron 33. A number of randomly generated binding domains was analysed by FACS with our screening approach. The best two were selected to buildup the complete repair PTM delivering the 3' exons. Transduction of type XVII collagen-deficient keratinocytes will verify the functionality of the selected PTM on RNA and protein level.

P068
Mechanisms for phenotypic variability in junctional EB: lessons for molecular therapies

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Recent pilot studies on causal therapies for genetic skin diseases have revealed that relatively small biological changes, e.g. moderately increased levels of a missing protein in the skin, can have substantial clinical effects. Epidermolysis bullosa (EB), a group of hereditary skin fragility disorders caused by mutations in the genes encoding components of the epidermal adhesion complex, has served as a prototype for such investigations. The junctional EB (JEB) subgroup is mostly associated with mutations in the genes for laminin 332 or collagen XVII (COL17A1) and characterized by trauma-induced tissue separation within the lamina lucida. The phenotypes associated with COL17A1 mutations range from mild to severe, and features like dystrophy or loss of nails, mucosal involvement, enamel defects and alopecia occur to a differing extent. Here, the systematic analysis of 34 different COL17A1 mutations in 43 patients with JEB-other revealed new insights into the phenotypic variability. We focused on the

effects of splice-site mutations, i.e. the nature and amounts of transcripts and polypeptides synthesized and their association with the phenotypic outcome. Careful molecular genetic and protein biochemical analysis revealed that even small amounts of collagen XVII have a remarkable effect on the phenotype. In contrast to complete null phenotypes, patients with only about 14% of collagen XVII of the control levels had clearly milder cutaneous involvement and a long life span. These data have significant implications for design of molecular therapies for JEB, since they suggest that a low degree of collagen XVII restoration may deliver substantial skin stability.

P069

Loss of kindlin-1 induces expression of cytokines in keratinocytes: model of pathogenesis of dermal fibrosis

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Kindler syndrome (KS) is a skin disorder caused by mutations in the FERMT1 gene encoding kindlin-1, an epithelial-specific phosphoprotein involved in β1 integrin activation. Early in life, KS manifests as a mechanobullous disease reflecting diminished cell adhesion, but many aspects of its later phenotypic features, including progressive poikiloderma and fibrosis of the skin and mucosa, remain unclear. Against this background, we addressed the pathogenesis of dermal abnormalities in KS by exploring cytokine profiles of KS keratinocytes and by characterizing KS skin fibroblasts *in vitro*, and by validating the findings *in vivo* in the skin of nine KS patients. We show that kindlin-1 deficient keratinocytes upregulate the expression ofIL-24, IL-20, transforming growth factor-β2 (TGF-β2), IL1F5, platelet derived growth factor B (PDGFB) and connective tissue growth factor (CTGF), and that KS fibroblasts exhibit an activated phenotype. These findings correlate with the presence of macrophages and of mediators of fibrosis, like α-smooth muscle actin, TGF-β1, IL-6 and CTGF, in KS skin. Based on these data we predict that mutations inFERMT1 gene cause epithelial cell stress and, as a stress response, secretion of cytokines that mediate local inflammation and fibrosis. The repeated cycles of epidermal cell stress, cytokine secretion, dermal inflammation and fibrotic processes underlie the phenotypic changes in different tissue compartments in the skin. Ourdata uncover cytokine-mediated paracrine cell communication processes as novel phenotype modulators in KS and thereby yield a new starting point for development of therapeutic strategies.

P070

No evidence of viral genomes in whole-transcriptome sequencings of three melanomas

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Several viruses are known to cause cancer, such as human herpes virus 8 in Kaposisarcoma and human papilloma viruses in cervical cancer. Recently, Merkel Cell Polyoma Virus (MCPyV) has been described in 80% of Merkel cell carcinomas (MCC). Similarly to MCC and Kaposi sarcoma, melanoma incidence is increased in immuno-suppressed patients. To address the question whether malignant melanoma might be caused by a so far unknown virus, we studied three melanoma metastasis samples by whole-transcriptome sequencing and digital transcriptome subtraction (DTS) analysis in order to detect viral sequences. None of the samples investigated harbored viral sequences. Artificial fusion transcripts between human and viral sequences added to the dataset as a positive control for the bioinformatics analysis, were detected. In conclusion, there is no evidence that viral infections play a role incutaneous melanoma development. To corroborate our results, we currently performa confirmation study in a larger set of melanomas.

P071

Skin compartments of different body areas harbour different amounts of mitochondrial deletions in aged wildtype mice

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Mutations of mitochondrial DNA play a causative role in aging of the skin including subcutaneous fat. We could previously show that the so called mitochondrial common deletion, a long-term marker for oxidative stress, is increasing in anage-associated manner in subcutaneous fat from the facial area of mice deficient in the repair of oxidative stress. Here, we investigated whether these mutations are also present in aged wildtype mice and whether reduction of subcutaneous fat is also associated with increased mutations of mitochondrial DNA at other body sites than the face. To address this question, skin samples of wildtype mice of different ages (3–4 months and 18–24 months) and different body areas (forehead, cheek, neck, shoulder, lower and medial back, breast and pads of the fore limbs and hind limbs) were taken, dissected in epidermal, dermal and subcutaneous fat compartments and subsequently screened for mitochondrial DNA deletions.

Dermal and epidermal compartments showed low levels of mitochondrial deletions and no significant age dependent increase of mitochondrial deletions in any investigated body areas. While epidermal and dermal compartments of all investigated body areas display only mild accumulation of mitochondrial deletions, there is an age dependant increase of the mitochondrial deletion in subcutaneous fat. This is particularly increased in the pads of the fore limbs compared to all other body sites. These results indicate that mitochondrial DNA deletions are increased in those areas of the body where prominent reduction of subcutaneous loss is observed in humans during the normal aging process.

P072

Whole genome (Exome) sequencing of Xeroderma pigmentosum patients reveals a finite and distinct pattern of mutations in pathophysiologically relevant genes

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Xeroderma pigmentosum (XP) is a rare autosomal recessive disease clinically characterized by photosensitivity, xerosis cutis, poikiloderma, telangiectasia and a1000-fold increased risk to develop skin cancer. Patients with XP are defective in nucleotide excision repair (NER) a mechanism responsible for removal of bulky helix-distorting DNA damage mainly induced by ultraviolet (UV) radiation. When UV-induced DNA damage is not removed from the genome, the remaining photoproducts will give rise to UV-signature mutations such as C to T and CC to TT transitions. While in XP patients it has been shown that isolated genes such as p53 harbor such mutations, thus far it was technically impossible to comprehensively investigate the exome of the whole human genome. In this study, we identified somatic mutations in DNA from two patients suffering from XP complementation group C. Skin samples of sun-exposed and non-exposed skin were compared to the respective germline genomes derived from blood of the patient. Exome-Sequencing was performed using sequence capture (NimbleGen EZ1, Nimble Gene Systems, Waldkraiburg, Germany) followed by next-generation sequencing.

Exome sequencing in PBCs enabled us to subtract the germline genome, leaving the somatic changes in XP. Although analysis are still under way, preliminary data demonstrate an over-representation of C>T and CC>TT transitions in skin cells (<500 in total).

This study indicates that even in DNA repair deficient tissues with a presumed mutator phenotype, only a finite number of somatic mutations is present in skin samples exposed to the relevant genotoxic stress. These findings could shed newlight on the relations of DNA mutations and cancer susceptibility.

P073

Vascular endothelial growth factor (VEGF) induces IL-23 expression in keratinocytes: a novel pro-inflammatory role for VEGF in psoriasis pathogenesis?

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Angiogenesis has an important role in tumor growth and metastasis. However, vascular remodeling also occurs in many inflammatory and autoimmune disorders, including the chronic inflammatory skin disease psoriasis. The pro-angiogenic vascular endothelial growth factor (VEGF) might also act as a pro-inflammatory factor in the skin in psoriasis as transgenic over-expression of VEGF in keratinocytes in mice results in skin inflammation and a phenotype resembling the human disease. At the same time, anti-VEGF treatment ameliorates skin inflammation in human psoriasis and mouse models of the disease. The mechanisms underlying the pro-inflammatory effects of VEGF are not known. In lesional skin in human psoriasis VEGF and the pro-inflammatory cytokine IL-23 are both strongly expressed by epidermal keratinocytes. To study the pro-inflammatory function of VEGF in psoriasis, primary human keratinocytes (NHEK) were transfected with a VEGF expression vector. VEGF overexpression in keratinocytes resulted in increased IL-23and IL-6 mRNA transcript abundance and protein expression at the same time. In order to dissect a possible link between VEGF and IL-23 expression protein phosphorylation profiles in lysates from cells over-expressing VEGF were investigated. VEGF overexpression in human keratinocytes strongly increased phosphorylation of p38α, CREB and HSp27. In sum, VEGF – which can be induced in keratinocytes by hypoxia and other cellular stresses – upregulates pro-inflammatory IL-23 and IL-6 secretion in keratinocytes via p38α, CREB and HSp27. Further studies are currently underway to investigate the exact mechanisms and define the proinflammatory effects of VEGF in psoriasis.

P074

Beta2 integrin-dependent release of oxygen radicals from macrophages is required for TGF-beta1 activation in cutaneous wound repair

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Patients suffering from Leukocyte Adhesion Deficiency Syndrome type 1 (LAD1) with impaired β2 integrin expression and function due to mutations in the gene encoding their common β chain (CD18) present with spontaneous skin ulcerations and severe wound healing disturbances.

In a model of full thickness excisional wounds we previously found that disruption of the β2 integrin signaling pathway in CD18-/-, Vav3-/- and Rac2-/- mice results in delayed wound healing. This is due to impaired formation of the phagocytic synapse between apoptotic neutrophils and macrophages leading to impaired oxidative burst and reduced release of active TGF-β1 at the wound site. However, the underlying mechanism is poorly understood.

We here investigated whether the β2 integrin-dependent release of ROS by macrophages upon phagocytosis of apoptotic neutrophils is responsible for TGF-β1 activation during wound healing. *In vitro* co-culture experiments of wildtype macrophages with apoptotic neutrophils induced high amounts of active TGF-β1 which were reduced by co-incubation with oxygen and nitrogen radicals scavengers. Notably, injection of the oxidative burst inducer Rotenone in wound margins of wildtype and CD18-/- mice enhanced the oxidative burst at wound sites and virtually rescued the wound healing defect of CD18-/- mice to wildtype levels.

These results suggest that TGF-β1 may be activated at wound sites by ROS and that in CD18 deficiency a reduced oxidative burst leads to reduced active TGF-β1 release and eventually to impaired wound healing. Modulation of the oxidative burst in wound margins may be a promising therapeutic approach for LAD1 patients but also to prevent fibrosis.

P075

AIM2 is overexpressed in psoriasis and an AIM2 inflammasome is active in human epidermal keratinocytes

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Although they are not professional immune cells, epidermal keratinocytes are at the first line of defense against invading pathogens and are able to initiate immune responses. In order to do so, they are equipped with danger sensors such as toll-like receptors and inflammasome components. Inflammasomes are cytoplasmatic multi-protein complexes that upon activation lead to the processing of the proinflammatory cytokine IL-1-beta. Only recently a novel inflammasome was characterized: The cytosolic protein 'absent in melanoma 2' (AIM2) mediates intracellular recognition of double stranded DNA (dsDNA) and subsequently triggers inflammasome activation in monocytes.

In this study we analyzed the activation of the AIM2 inflammasome in human epidermal keratinocytes and its potential role in inflammatory skin diseases. We found increased AIM2 expression in lesional psoriatic skin compared to healthy or non-lesional skin. Additionally, active caspase-1 and IL-1-beta production demonstrated inflammasome activity in lesional psoriatic plaques. *In vitro*, human epidermal keratinocytes secreted IL-1-beta and active caspase-1 indicating inflammasome activity upon stimulation with dsDNA. IL-1-beta release was abrogated when AIM2 was blocked or dsDNA was pretreated with DNase. *In vivo*, cutaneous expression of IFN-gamma correlated with the expression of AIM2 in psoriatic skin and *in vitro* IFN-gamma increased AIM2 in primary epidermal keratinocytes. Finally, DNA stainings indicated the presence of cytosolic DNA in keratinocytes of lesional psoriatic skin as a possible trigger of AIM2 activation *in vivo*.

These data suggest that cytosolic DNA activates an AIM2 inflammasome in human epidermal keratinocytes triggering the secretion of IL-1-beta. As AIM2 expression is high in the epidermis in psoriasis and cytosolic DNA can be detected in lesional psoriatic plaques AIM2 activation might be a critical contributor to cutaneous inflammation in this chronic disease.

P076

Treg deficient in IL-10 are insensitive to activation by ATP *in vivo* and fail to suppress contact hypersensitivity reactions

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Injection of CD4+CD25+Foxp3+ regulatory T cells (Treg) into TNCB-sensitized mice before challenge suppresses the elicitation phase of murine contact hypersensitivity (CHS) reactions. In contrast, Treg deficient in IL-10 production (IL-10-/- Treg) fail to do so. Because we have recently shown that adoptively transferred wild type (WT) Treg normally become activated *in vivo* by ATP resulting in production of immunosuppressive adenosine and suppression of the CHS response, we analyzed the activation status and the adenosine production of IL-10-/- Treg in comparison to WT Treg. We found that IL-10-/- Treg were not able to become activated by graded doses of ATP *in vitro* (as indicated by reduced expression of CD69 and CD44) and produced significantly reduced amounts of adenosine as compared to WT Treg. As a functional consequence IL-10-/- Treg were unable to suppress the adherence of CD4+ effector T cells to endothelial cells *in vitro*, explaining their inability to suppress the ear swelling reaction *in vivo*. When analyzing the intracellular signal transduction of the ATP receptor P2X7 in Treg, we found significantly reduced calcium levels in IL-10-/- Treg as compared to WT Treg after stimulation with ATP. Thus, IL-10-/- Treg have a defect in reacting to ATP and in producing adenosine, and our data indicate that production of IL-10 by Treg is dispensable for the suppression of CHS responses. Moreover, these data have to be taken into account when using IL-10-/- Treg in assessing the contribution of IL-10 to the Treg-mediated suppression in other disease models.

P077

Formation of aggregates between regulatory T cells and dendritic cells during the suppression of immune responses are guided by adenosine

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During the sensitization phase of CHS reactions intravenously injected murine CD4+CD25+Foxp3+ regulatory T cells (Treg) migrate into the lymph nodes (LN) and engage in close contacts to dendritic cells (DC). These DC-Treg aggregates involve gap junctions and as a consequence thereof, DC are less mature (decreased CD80 and CD86 expression) and less stimulatory as analyzed by antigen presenting assays. Therefore, when sensitizing animals with TNCB after injection with Treg and challenging them according to standard protocols, the ear swelling reaction is abrogated. To further elucidate the underlying mechanisms of the DC-Treg aggregates, we set up co-cultures of DC and Treg *in vitro*. Using video microscopy we show formation of large DC-Treg clusters within 2 h. In contrast, DC established hardly any stable contacts with polyclonal CD4+ T cells. Life images sampled over 2 h demonstrated fast movement of the DC in the co-cultures, resulting in frequent contacts with the rather immobile Treg. Next we analyzed the role of adenosine, which is produced by the Treg via the ectonucleotidases CD39 and CD73, during the aggregation of DC and Treg. We detected that inhibition of adenosine production by the CD39-blocking agent POM-1 abolished the formation of clusters between DC and Treg significantly. In contrast, addition of exogenous adenosine to the co-culture of CD4+ T cells and DC leads to the formation of clusters comparable to the Treg-DC aggregates.
Thus, our data indicate that adenosine produced by Treg is essential for the formation of aggregates between DC and Treg, which is required for the immunosuppressive action exerted from Treg on DC.

P078

Human 6-sulfo LacNAc (slan)-expressing dendritic cells exhibit strong reactivity to inflammasome stimuli

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Inflammasomes are cytosolic sensors of innate immune cells that are able to recognize the presence of a large and diverse set of Danger-associated molecular patterns (DAMPs). The outcome of DAMP recognition is autocatalytic activation of caspase-1 within the inflammasome complex which in turn leads to the conversion of the inactive pro-IL-1 beta into its active and secreted form. 6-sulfo-LacNAc expressing DCs (slanDC), a highly proinflammatory subset of human blood DCs that has been previously described by our group, serve as the major and early source of IL-12 and TNF-alpha in response to LPS and can become inflammatory dermal dendritic cells in psoriasis. SlanDC are able to produce high amounts of IL-1 beta following stimulation with various TLR ligands and are in this respect superior incomparative studies with monocytes and CD1c+ DC. Furthermore, slanDC have been shown to promote the polarization of T cells into proinflammatory IFN-gamma-producing Th17 cells. Therefore, we asked whether slanDC's potency of producing high amounts of secreted IL-1 beta is linked to the activation of inflammasomes within these cells what we worked out by applying a set of known activators of inflammasomes to matured and LPS-primed slanDC. The priming was done with increasing concentrations of ultrapure LPS (1, 10, 100, 1000 ng/ml) what on its own led to no prominent secretion of IL-1 beta. In contrast, a highly elevated secretion of IL-1 beta could be observed after adding ATP (0.8 and 2 mM), nigericin (5 and 10 M) and muramyl dipeptide (10 and 100 g/ml) while these stimuli alone did not lead to IL-1 beta secretion. In the case of ATP we show that a certain threshold of ATP concentration (approximately 600 M) has to be surpassed to induce IL-1 beta secretion. For ATP and nigericin the effect appeared to be accompanied and dependent on an efflux of K+ ions as adding increasing concentrations of extracellular K+ led to concentration-dependent inhibition of the elevated secretion of IL-1 beta with complete inhibition approaching physiologic concentrations. We conclude that slanDC can be effectively stimulated to secrete IL-1 beta after inflammasome activation. As these cells have been shown to be present under certain inflammatory conditions such as psoriasis and can effectively stimulate aTh17 polarization of T cells our data add important aspects of slanDC's role in bridging innate and adaptive immunity.

P079

Different ways for controlling proinflammatory slan (6-sulfo Lac NAc) dendritic cells

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Chronic inflammatory skin diseases such as psoriasis and atopic dermatitis are considered to be the result of proinflammatory DCs stimulating inflammatory T cell responses. However the identity and the modulation of these dendritic cells are still incompletely understood. We identified slanDCs as a population of TNF-α, iNOS producing inflammatory DCs in psoriasis and observed increased frequencies of these cells also in atopic dermatitis. In blood, slanDCs are the largest population of native human DCs. Freshly isolated slanDCs are immature, but show a spontaneous maturation *in vitro* within 6 h. In the immature state they are capable of producing high levels of TNF-α, but fail to produce IL-12 or IL-23. Mature slanDCs are a rich source of the proinflammatory cytokines IL-23, IL-12, IL-1β, IL-6 and drive strong Th17- and Th1- responses. In this study, we asked for the flexibility of the pro-inflammatory capacity of slanDCs when coming into contact with defined micromilieu factors. For these experiments native slanDCs were isolated by magnetic cell sorting from buffy coats of healthy donors to a purity of >95%. Immature slanDCs were treated by IL-10, PGEs or were repeatedly treated with LPS to induce endotoxin-tolerance (ET). IL-10 treatment of freshly isolated slanDCs induced an inhibition of their phenotypic maturation, as revealed by a deficiency to upregulateCD83, CD86, HLA-DR as well as a failure to produce TNF-α and IL-12 production when stimulated with LPS. In the presence of PGE2 and similarly in the presence of cAMP-analoga, slanDCs displayed a strong capacity to undergo phenotypic maturation, however, their production of IL-12 and TNF-α was inhibited while the IL-10 production was increased. In addition, we studied the effects of ET in slanDCs. For these experiments native slanDCs were immediately treated with different doses of LPS (0.1–100 ng/ml) and stimulated after 12 h with a second doses of LPS (100 ng/ml). The initial low level LPS challenge led to a dose-dependent reduction of TNF-α production as revealed by ELISA and intracytoplasmatic cytokine staining on the single cell level. Interestingly, ET slanDC showed signs of an increase in maturation. Taken together the proinflammatory capacity of slanDCs, previously demonstrated in psoriasis, is profoundly and differently modulated by different micromilieu factors as demonstrated for IL-10, PGE2 and ET.

P080

Pro- and anti-inflammatory signalling cascades synergize to induce an M2-liketumor macrophage-associated activation pathway involving the novel CD20 homolog Ms4a8a

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Tumor-associated macrophages (TAM) represent alternatively activated (M2) macrophages that support tumor growth. Gene profiling of a stabilin-1+, LYVE-1+ M2 macrophage subset identified Ms4a8a as a novel TAM molecule induced in bone marrow-derived macrophages (BMDM) by combining M2 mediators (IL-4, glucocorticoids) and tumor-conditioned media (TCM). *In vivo*, Ms4a8a was expressed by TAM in mammary carcinoma and malignant melanoma. In macrophage-like RAW264.7 cells, forced over-expression of Ms4a8a activated a special gene expression program (Tcfec, Sla, Spink5) co-regulated by LPS. In Ms4a8a+RAW264.7 cells, LPS further mediated induction of histidine decarboxylase paralleled by enhanced production of histamine. In BMDM, M2 mediators and LPS synergized to induce Ms4a8a itself and its target genes. LPS exerted its effects via TLR4, MyD88, NFB and p38MAPK. TCM did not engage in TLR signalling, but directly activated p38. Anti-inflammatory signalling was similarly indispensable for induction of Ms4a8a as shown by using glucocorticoid receptor dimerization-deficient BMDM. Despite induction by pro- and anti-inflammatory signalling, Ms4a8a+ BMDM wereM2-like macrophages exhibiting strong arginase expression, and enhanced the growth of subcutaneous transplant tumors *in vivo*. In conclusion, Ms4a8a acts to fine tune macrophage immune responses. Synergy between pro- and anti-inflammatory signalling cascades favors a concept of macrophage plasticity beyond the M1/2 dichotomy.

P081

Repression of cAMP up-regulation disarms human Tregs

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Regulatory T cells (Tregs) contribute to the therapeutic resistance of progressive melanoma by impairing protective effector T cell responses. Functional inactivation of Tregs in melanoma patients therefore seems to represent a useful strategy to enhance effectiveness of therapeutic vaccination. However, due to the lack of exclusive Treg markers, current attempts to reduce Treg activity are based on non-specific depletion strategies that interfere with the clonal expansion of tumor antigen specific T lymphocytes.
We recently discovered that Tregs accumulate the second messenger cyclic adenosine monophosphate (cAMP) upon activation. Here we demonstrate that repression of cAMP up-regulation by promoting its degradation or by inhibiting denovo synthesis abrogates the suppressive activity of human Tregs both *in vitro* and in a humanized mouse model *in vivo*. Contrariwise, repression of cAMP production improves effector T cell function. Thus cAMP repression works to the same direction by impairing Tregs and improving T effector cells at the same time. These results disclose cAMP repression as an attractive therapeutic strategy to increase the efficacy of anti-tumor vaccination in melanoma patients.

P082

Prolonged removal of autoreactive, but not total, IgE by immunoadsorption in patients with atopic dermatitis

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Recently, we have shown that immunoadsorption (IA) leads to significant clinical improvement of patients with refractory atopic dermatitis (AD) and high levels of total serum IgE by temporal depletion of circulating and prolonged removal of skin-bound total IgE as well as reduction of dermal inflammatory infiltration comprising CD3+,CD4+, CD1a+, and HLA-DR+ cell numbers. To further investigate the mode of action of IA in AD and following few reports on the presence of autoreactive serum IgE antibodies correlating with severity of AD, we attempted to confirm the presence of IgE autoreactivity in AD patients. In addition, we tested if IA leads to a reduction of IgE autoantibodies in these patients. The direct effect of treatment on IgE antibody reactivity to human proteins has so far been described in only one patient with AD receiving systemic cyclosporin A. However, the impact of directly targeting IgE either by IA or omalizumab on IgE autoreactivity has not yet been investigated. Pre- and post-treatment sera from 10 patients with refractory AD and total serum IgE levels >4500 kU/l were analyzed for IgE autoreactivity by immunoblotting (IB) using human epidermal extracts. Results were correlated with scoring atopic dermatitis (SCORAD) and total serum IgE values before and after IA. Nine of 10 AD patients, but not healthy individuals, showed serum IgE autoreactivity by IB. In contrast to total IgE levels, a significant decline in autoreactive IgE, as shown by reduced number and intensity of positive bands by IB ($P = 0.004$ and $P = 0.028$, respectively), as well as significant correlation between IgE autoreactivity and SCORAD was observed 13 weeks after initiation of IA ($P = 0.002$, $r = 0.673$). Our data show that clinical improvement of AD patients is paralleled by prolonged depletion of autoreactive, but not total, IgE, thereby supporting a clinical role of IgE-mediated reactivity to self antigens. The mechanisms of prolonged depletion of autoreactive IgE antibodies following IA still need to be defined but may involve (i) impaired exposure of autoreactive B cells to autoantigens due to prolonged removal of skin-bound total IgE and (ii) reduced scratching observed after IA, less tissue damage, and ultimately, attenuated release of autoantigens from the skin. We therefore assume that AD patients with autoreactive IgE may preferentially benefit from IgE-targeted the rapies like IA or omalizumab.

P083

Analysis of the tolerogenic properties of two subpopulations of humanIL-10-modulated dendritic cells

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Our previous studies demonstrated that IL-10-modulated tolerogenic human dendritic cells (IL-10DC) induce anergic regulatory CD4+ T cells. By flow cytometry analyses, two subpopulations of IL-10DC were identified with different maturation states. Here, we characterized these two populations of human tolerogenic IL-10DC in detail with regard to their phenotype and their capacity to generate anergic CD4+ T cells. For this purpose, we compared the expression of costimulatory molecules of the B7- and ILT-family and of chemokine receptors (CCR7, CXCR4, CXCR5) between human mature DC (mDC) and IL-10DC. In addition, both IL-10DC subpopulations were purified by FACS sorting for coculture experiments with naive CD45RA+CD4+CD25high-CD45RO- T cells. As compared to mDC, we observed an impaired upregulation of CD83, CCR7 and B7-H2 and an upregulation of ILT-4 on IL-10DC. The experiments revealed the existence of two subpopulations of IL-10DC with distinct states of maturation, characterized as CD83highCCR7highHLA-DRhigh and CD83lowCCR7lowHLA-DRlow IL-10DC. In contrast to CD83, the moleculeCCR7 is not involved in T cell activation and, therefore, was used in further experiments as surface molecule for DC sorting. We are capable of separating CCR7+HLA-DRhigh and CCR7-HLA-DRlow IL-10DC subsets with a purity of >95%.Notably, restimulation experiments (using anti-CD3/anti-CD28-mAb) showed that both induced T cell populations exhibited the features of anergic T cells demonstrated by a significantly reduced T cell proliferation. In conclusion, both CD83highCCR7highHLA-DRhigh and CD83lowCCR7lowHLA-DRlow IL-10DC subpopulations display properties of tolerogenic human DC which may be used for the development of novel therapeutic approaches for allergies, autoimmune disease or transplant rejections.

P084
Regulation of Th17 T-helper cell differentiation by beta-2 integrinCD18

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CD18 is the common beta-chain of $\beta 2$ integrins that are important for transendothelial migration of leukocytes as well as for T-cell activation during antigen presentation. LFA-1 (CD11a/CD18) is the only $\beta 2$ integrin expressed on T-cells, and triggering of LFA-1 is required for full activation and Th1 differentiation. In addition, contribution of LFA-1 for differentiation of regulatory T cells (Treg) has been demonstrated.

We used T-cells from CD18-deficient (CD18-/-) mice to examine the role of LFA-1 in plasticity of T-helper cell differentiation, especially towards the recently described T-helper cell types Th17 and Th9. We isolated T-cells from spleens of CD18-/- mice and stimulated these with antigen-presenting dendritic cells (DC) as well as with antibodies against CD3 and CD28. Subsequently, we evaluated T-cell differentiation by analyzing cytokine production. In addition, we determined expression of T-helper cell-specific transcription factors employing RT-PCR of isolated RNA.

Compared to wild-type controls, CD18-/- T-cells produced significantly more IL-17 upon stimulation. In contrast, secretion of (former Th2 cytokine) IL-9 of CD18-/- T-cells was significantly less compared to IL-9 production of WT T-cells. Analyzing transcription factors T-bet, Gata-3, Foxp3, RORγt, and PU.1, revealed that CD18-/-T-cells are shifted to Th17 differentiation already, while Th9 differentiation is impaired at the same time.

We here show for the first time that LFA-1 is required for production of IL-9 by T cells, and that Th17 differentiation is regulated by CD18.

P085 (V23)
Interferon-alpha abrogates suppressor activity of human CD4+CD25high regulatory T cells *in vitro*

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Interferon-alpha (IFN-α) represents the only approved adjuvant therapeutic approach in stage Ib-III melanoma patients and pleiotropically affects tumor cells as well as the immune system. In our study, we investigated the effect of IFN-α on the function of human naturally occurring CD4+CD25high regulatory T cells (Tregs). Tregs as well as CD4+CD25low effector T cells (Teffs) were isolated from buffy coats and cocultured in suppressor assays in the presence of irradiated allogeneic PBMC as antigen presenting cells (APC) and soluble anti-CD3-mAb. As reported previously, Tregs inhibited the proliferation of Teffs up to 90%. Notably, addition of IFN-α promoted a significant impairment of the suppressor activity resulting in an increased T cell proliferation as compared to controls. In order to evaluate the target cell population of IFN-a, pre-incubation experiments of PBMC, Teffs and Tregs with IFN-α were done. Pre-treatments did not alter the induced T cell suppression in the subsequently performed suppressor assays, suggesting that the effect of IFN-α is dependent on its presence during the interaction of Teffs, Tregs and APC. To analyse the role of APC as targets of IFN-a, suppressor assays of Teffs and Tregs were alternatively activated by anti-CD3 and anti-CD28 in the absence of PBMC. Here, addition of IFN-a resulted in a significant inhibition of the Treg-induced suppression as described for PBMC/anti-CD3-mAb-induced control experiments, excluding APC as IFN-a targets. IFN-a treatment was followed by a pronounced shift in the cytokine pattern in the suppressor assays (decreased secretion of IL-13, augmented release of IL-10 and IFN-gamma) that might be involved in the abrogation of Treg-induced suppressor activity. Our study demonstrate that IFN-α abolished the suppressor properties of human Tregs independent from APC-mediated effects. These properties of IFN-α on Treg-induced suppression *in vitro* may be relevant for modulation of tolerance processes *in vivo* and its capacity to act as an effective therapeutic approach in cancer and infection.

P086
Induction of targeted cell migration by cutaneous administration of a DNA vector encoding a biologically active chemokine CCL21

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Skin inflammation can induce local expression of CCL21, which is subsequently drained to lymph nodes (LNs) influencing their cellular composition. To determine whether the same can be achieved by dermal administration of a plasmid DNA (pDNA) encoding CCL21, we generated a pDNA-based gene construct allowing high-level expression of CCL21. Expression and secretion of biologically active CCL21 were confirmed *in vitro* by immunohistochemistry, western blot analysis, ELISA, and transwell chemotactic assays. *In vivo* experiments showed cellular expression of transgenic CCL21 after particle-mediated gene gun delivery of pDNA into skin. CCL21 was expressed in the epidermis, consequently secreted into the upper dermis, and transported into the draining LNs, which resulted in increased CCL21 concentration, total cell number, and frequencies of CD11c(+) DCs and CD4(+)/CD62L(+) nave, CD4(+)/CD62L(-), and CD8(+)/CD62L(-) effector memory T-cells (expressing CCL21 receptors CCR7 or CXCR3), as well as retention of adoptively transferred T-lymphocytes, in the draining LNs of plt/plt mice (lacking endogenous expression of CCL21). Our studies show that biologically active CCL21 can be overexpressed by genetic means *in vitro* and *in vivo*. This strategy allows reconstitution of a genetic defect and colocalization of different cell types in the secondary lymphoid organs, an important prerequisite for targeted cell migration.

P087 (V10)
An important role of RANK-RANKL signaling during skin carcinogenesis

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The development of skin cancer seems to be controlled by the immune system and innate as well as adaptive immune responses are crucially involved in the regulation of tumor growth. In a transgenic mouse model (K14-RANKL tg) we have shown that cutaneous over-expression of RANK ligand (RANKL, CD254) resulted in the peripheral expansion of regulatory T cells (Tregs) via interaction with RANK-RANKL activated Langerhans cells. Since Tregs are potent suppressors of anti-tumoral immunity, we investigated the relevance of RANKL signaling during carcinogenesis. Surprisingly, in a two-stage chemo-carcinogenesis study K14-RANKL tg mice developed significantly fewer skin tumors compared to wildtype controls. Moreover, the tumor growth was reduced in K14-RANKL tg mice and most of the tumors were rejected within several days whereas skin tumors grew progressively in wildtype mice pointing to increased anti-tumoral immune responses in K14-RANKL tg mice. The reduced skin tumor development and tumor growth in tg mice could be attributed to increased RANK-RANKL signaling since blocking this pathway using the specific antagonist RANK-Fc led to a higher frequency of tumors per mouse and an accelerated tumor growth compared to K14-RANKL tg mice treated with control IgG. Histology of tumor tissue demonstrated highly differentiated cells in wildtype tumors suggesting the development of squamous cell carcinomas while tumors of K14-RANKL tg mice presented as small low differentiated papillomas. As expected, flow cytometry of tumor draining lymph nodes revealed increased numbers of Tregs in K14-RANKL tg mice compared to wildtype controls leading to the speculation that potent anti-tumoral immune responses might overcome the Treg mediated systemic immunosuppression in tg mice. Interestingly, in tumor draining lymph nodes of K14-RANKL tg mice the total numbers of CD8+ T cells as well as the levels of CD8+ cytotoxic T cells (CTL) were increased compared to wildtype mice and additionally, these CTL expressed higher levels of activation and cytotoxic markers such asCD43, granzyme B, IFN-gamma, or the activating CD94 receptor (NKG2B/D). Since during skin carcinogenesis cutaneous antigen presenting cells migrate from the epidermis to regional lymph nodes and induce the differentiation and activation of anti-tumoral effector cells and since RANK-RANKL signaling has been shown to increase cell viability we analyzed the numbers and function of antigen presenting cells in tumor draining lymph nodes. Indeed, antigen presenting cells fromK14-RANKL tg mice showed a prolonged life-span and higher viability suggesting an increased T cell stimulatory capacity. This increased T cell stimulatory capacity might possibly explain the up-regulated numbers of CTL in regional lymph nodes fromK14-RANKL tg mice compared to controls. Taken together, our data indicate that RANK-RANKL signaling seems to play an important role for the differentiation and activation of CTL via up-regulating the viability of cutaneous antigen presenting cells thus favouring rejection of tumors.

P088
Dendritic cells from mice with heterozygous deficiency of manganese superoxide dismutase (SOD2) have features of aged dendritic cells

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Dendritic cells (DC) are central in regulating skin immunity. Immunosenescence is associated with a chronic inflammatory state. Little is known about the contribution of DC to 'inflamm-aging'. When determining Langerhans cell (LC) numbers, we found a 60% reduction of LC in aged epidermis. Reactive oxygen species are linked with aging. The mitochondrial manganese superoxide dismutase (SOD2) is in the first line of antioxidant defence. We investigated the function of DC from SOD2 heterozygous mice (SOD2+/−) and found that at 4 months of age LC numbers are not altered, but activated LC have impaired expression of MHC-II and CD86. Immature SOD2+/− DC produced increased proinflammatory IL-6 and chemokines CXCL1 and CXCL2. When activating SOD2+/− DC by LPS they less efficiently upregulated MHC-II and CD86. Surprisingly, *in vivo* contact hypersensitivity (CHS) was enhanced in SOD2+/−mice although SOD2+/− DC were less potent in stimulating wt-T cells. However, SOD2+/− T cells showed increased proliferation, even when stimulated with SOD2+/−DC, possibly explaining the increased CHS. Our findings suggest that SOD2 loss during aging is a molecular candidate in the regulation of 'inflamm-aging' conveying both immunosuppressive and proinflammtory signals through alteration of DC and T cell functions.

P089
Disruption of the epidermal barrier induces regulatory T cells in a Langerhans cell-dependent fashion

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Ultraviolet radiation (UVR) 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 UVR, as demonstrated for the release of antimicrobial peptides (AMPs), thereby protecting the skin against microorganisms. Recently, we observed that AMPs contribute to photoimmunosuppresssion via induction of regulatory T cells (Treg). Since disruption of the epidermal barrier induces AMPs and since UVR disrupts the epidermal barrier, we asked whether disturbance of the epidermal barrier by itself can also suppress the adaptive immune response via induction of Treg. Therefore, C57BL/6 mice were tape stripped on their shaved backs before sensitization with 2,4-dinitrofluorobenzene (DNFB). After 5 days ear challenge was performed and the contact hypersensitivity (CHS) reaction was measured. Application of DNFB onto tape stripped skin did not result in sensitization, but induced Treg as demonstrated by adoptive transfer experiments. Furthermore, tape stripping increased the number of apoptotic antigen presenting cells (APCs) in the draining lymph nodes. This suggests that barrier disruption like UVR might damage epidermal APCs and thereby induce Treg. The crucial role of Langerhans cells in the induction of Treg was demonstrated by using langerin diphtheria-toxin receptor knock-in mice (LDTR). The reduction of CHS response upon tape stripping was lost after depletion of langerin positive cells in LDTR mice treated with diphtheria-toxin (DT). Additionally, the CHS response was not reduced in wild type recipients upon adoptive transfer of lymphocytes obtained from tape stripped and DT injected LDTR donors, indicating that the induction of Treg upon tape stripping is Langerhans cell dependent. Disruption of the epidermal barrier enables the penetration of allergens and the induction of inflammation. Hence, the induction of tolerance via generation of Treg upon barrier disturbance may be part of a compensatory protection mechanism avoiding excessive sensitization to environmental agents.

P090
Vitamin D is required for T cell mediated antimicrobial activity of human macrophages

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It is widely accepted that acquired T cell responses are critical to host defense against microbial pathogens, yet the mechanisms by which they act in humans remain unclear. Here we demonstrate that T cells by the release of IFN-γ induce a vitamin D dependent upregulation of antimicrobial peptides, autophagy and phagosomal maturation. Together this provides an acquired immune mechanism for overcoming the ability of intracellular pathogens to block phagosome maturation and evade macrophage microbicidal killing. By using cultures with vitamin D sufficient serum IFN-γ activation directly induced an antimicrobial activity against intracellular infection. These findings indicate that vitamin D is required for human T cell mediated immunity to microbial infection.

P091
Potential psoriatic autoantigens may result from cross-reactive streptococci-specific immune-responses

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Psoriasis is an HLA-Cw6-associated T-cell mediated autoimmune disease of the skin often triggered by streptococcal angina. To identify psoriatic autoantigens based on molecular mimicry we compared the reactivity of sera from psoriasis patients and rabbits which had been immunized with heat-killed *S. pyogenes*. When tested with keratinocyte lysates fractionated by 2D-gel electrophoresis, common serological reactivities of patients and streptococci-exposed rabbits included the proteins keratin 6, ezrin, maspin, peroxiredoxin 2 and Hsp27. When used for stimulation of peripheral blood lymphocytes these proteins induced an increased T-cell activation in psoriasis, which was particularly evident for HLA-Cw6-positive patients. T-cell lines expanded by repetitive stimulation with maspin, ezrin, peroxiredoxin 2 or Hsp27 consisted predominantly of CD8-positive T cells and employed T-cell receptor β-chain rearrangements, which were highly homologous to those found within the corresponding skin lesion. Several immunodominant epitopes on the proteins could be defined according to sequence alignments with the whole genome of *S. pyogenes* or predicted HLA-Cw6 anchor positions. These data indicate that maspin, ezrin, peroxiredoxin 2 and Hsp27, but also keratin 6 may act as potential autoantigens of across-reactive streptococcal-induced autoimmune response in psoriasis.

P092
Single cell analysis confirms antigen-specific clonal T cell expansions within psoriatic skin lesions

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Today, psoriasis is considered as a T-cell mediated autoimmune disease. A clonal antigen-specific T-cell expansions of the lesional psoriatic immune response has been proposed by T-cell receptor (TCR)-analysis of the inflammatory infiltrate in psoriatic skin lesions.

To define the clonal nature of the lesional psoriatic T-cell response we developed an RT-PCR-based method to characterize the paired TCRα- and β-chain rearrangements from single T cells, which had been were isolated from lesional psoriatic biopsies using magnetic beads coated with CD4- or CD8-monoclonalantibodies.

Single cell TCR analysis identified multiple CD4+ or CD8+ T cells with identical TCR rearrangements in both epidermal and dermal lesional psoriatic T cell preparations from HLA-Cw6-positive psoriasis patients, with a somewhat stronger clonal expansions in the CD8+ T cell compartment. Paired $\alpha\beta$ TCR rearrangements from clonal CD8+ T cells were employed to generate recombinant TCR hybridomas using the TCR deficient mouse T hybridoma cell line 58 α-/β-. Hybridomas expressing $\alpha\beta$TCRs from lesional psoriatic T-cell clones reacted selectively against HLA-Cw6-transfected but not HLA-Cw6-negative cells of the keratinocyte cell line, HaCaT.

Our results prove the clonal nature of the lesional psoriatic T-cell response on a single cell level and suggest that the psoriatic autoimmune response is directed against a keratinocyte protein, which is presented by HLA-Cw6 to the immune system.

P093
Enhanced primary but not memory anti-viral immune responses by cutaneous over-expression of RANKL

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Cutaneous infections are controlled by the immune system. Since the RANK-RANKL interaction is crucially involved in dendritic cell – T cell communication as well as the regulation of immunity we investigated whether RANK-RANKL signaling might play a role in cutaneous anti-viral immune responses. Therefore, transgenic mice over-expressing RANKL under control of the keratin-14 promoter (K14-RANKL tg) were epicutaneously infected with Herpes simplex virus type I (HSV). Interestingly, K14 RANKL tg mice developed significantly smaller skin lesions compared to wildtype controls and, moreover, the number of virus particles was reduced in tg versus wildtype skin as evidenced by immunofluorescence staining and quantitative real time-PCR suggesting increased primary anti-viral immune responses in K14RANKL tg mice. Since during the induction of cutaneous anti-viral immune responses antigen presenting cells migrate from the skin to regional lymph nodes and induce the differentiation of virus-specific effector T cells and since dendritic cells stimulated via RANK-RANKL signaling have been shown to expand virus-specific CD8+ cytotoxic T cells we analyzed the numbers and function of CD8+ T cells in regional lymph nodes. Flow cytometry revealed increased levels of total CD8+ T cells as well as an up-regulated IFN-gamma and granzyme B secretion in CD8+ T cells fromK14-RANKL tg compared to wildtype mice 8 days after primary HSV-infection. To assess whether RANK-RANKL signaling also affects the development of CD8+ memory T cells we performed re-infections of K14-RANKL tg and wildtype mice 10 weeks after the first HSV challenge. Of note, the skin lesions in re-infected mice are normally smaller compared to the primary infection. However, we did neither observe differences in skin lesion size between re-infected K14 RANKL tg and wildtype mice nor in the number of virus particles in the epidermis or dermis. Furthermore, we detected comparable levels of CD62LhighCCR7+ central and CD62LlowCCR7-effector memory T cells in lymphoid as well as peripheral tissues from K14-RANKL tg and wildtype mice suggesting that RANK-RANKL signaling plays a rather minor role for the differentiation of CD8+ memory T cells. Together, these data indicate that RANK-RANKL signaling might be crucially involved in cutaneous MHC class I mediated anti-viral immune responses during primary infection. In contrast, RANK-RANKL interactions seem to be of ancillary importance for the development of anti-viral memory responses.

P094
Thy-1 (CD90) regulates extravasation of inflammatory cells during inflammation

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Human Thy-1 (CD90) has been shown to mediate adhesion of inflammatory cells to activated microvascular endothelial cells via interaction with Mac-1 *in vitro*. Since there are no data showing the physiological relevance of Thy-1 for the recruitment of inflammatory cells *in vivo* different inflammation models were investigated in Thy-1-deficient mice and wild type mice.

In thioglycollate-induced peritonitis the number of neutrophils and monocytes was significantly diminished in Thy-1-deficient mice. During acute lung inflammation the extravasation of eosinophils and monocytes into the lung was significantly reduced in Thy-1-deficient mice. Moreover, during chronic lung inflammation the influx of eosinophils and monocytes was strongly decreased in Thy-1-deficient mice. These effects were independent on Thy-1 expression on T cells shown by reconstitution of bone marrow of Thy-1-deficient mice with wild type bone marrow. In spite of the strong Thy-1 expression on T cells in chimeric mice the extravasation of inflammatory cells was significantly diminished compared to control mice. Finally, the altered number and composition of infiltrating leukocytes in Thy-1 deficient mice modified the chemokine/cytokine and protease expression at the site of inflammation. In conclusion, Thy-1 contributes to the control of the recruitment of inflammatory cells and thus is involved in conditioning the inflammatory microenvironment.

P095
Linking ROS production and HA degradation – a crucial role for the generation of endogenous ligands in CHS responses to contact sensitizers

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Analogous to anti-infectious responses, contact allergens trigger innate immune and stress responses involving pattern recognition receptors and production of reactive oxygen species. Understanding the underlying molecular mechanisms is a crucial pre-requisite for the development of reliable *in vitro* test systems for the identification of chemicals with skin sensitizing potential. Moreover, modulation of these responses will help to prevent the inflammation that is crucial for the sensitization to contact allergens and should, therefore, result in new therapies for allergic contact dermatitis. In the mouse contact hypersensitivity (CHS) model we have previously shown a role for the Toll-like receptors TLR2 and TLR4. Thus our present study aimed at the analysis of putative endogenous ligands such as hyaluronic acid fragments for these TLR as well as the role of oxidative stress responses in the pathogenesis of CHS.

Induction of ROS and their role in the CHS response was identified by measurement of ROS production in dendritic cells *in vitro* and in murine skin *in vivo*. Antioxidants and hyaluronidase inhibitors were used to analyse the role of ROS and HA degradation *in vitro* and *in vivo*. Generation and degradation of hyaluronic acid as a result of ROS formation was studied in the skin by immunohistology.

We demonstrate a role for ROS production after contact allergen stimulation and its potential influence on the oxidative degradation of hyaluronic acid. Furthermore we provide evidence for the indirect activation of TLRs by contact allergen induced production and degradation of hyaluronic acid in the inflammatory skin milieu. In the CHS model we demonstrate the *in vivo* potential of inhibitors of hyaluronic acid metabolism and of anti-oxidants to prevent CHS responses when used in a short time window before or after sensitization and elicitation.

Innate immune receptor signaling can be indirectly induced by contact allergens either by production or release of endogenous danger signals in the skin microenvironment. Here we demonstrate analogies between innate immune and stress responses to contact allergens and infections. Here we show a direct link between ROS production and breakdown of extracellular matrix components indirectly triggering TLRs. Taken together we point out future strategies for causative therapies of allergic contact dermatitis by targeting innate immune and stress responses.

P096
Tolerance Induction towards Type XVII Collagen

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The induction and maintenance of tolerance towards a neo-antigen is critical to the success of gene therapy in autosomal recessive genetic diseases. One such disease is junctional Epidermolysis bullosa, in which patients lack type XVII collagen in the dermo-epidermal basement membrane zone. Hence, the aim of this study was to investigate novel protocols that prevent autoimmunity towards type XVII collagen. Our approach involved the induction of tolerance by targeting dendritic cells (DC), which are able to induce antigen-specific regulatory T cells. As a model antigen we used the immunodominant domain of human type XVII collagen, NC16A. We fused this domain to the single chain variable fragment antibody (scFv) specific for murine DEC205 (DEC-NC16A), which is expressed on a subset of DCs. NC16A fused to a single chain isotype control, as well as scFv against DEC205 alone served as control groups. After *in vivo* transfection of C57BL/6 mice, the expression as well as binding capacity of scFv-DEC-NC16A was tested in stainings of draining lymph nodes. Immune suppression was investigated in a model, which mimics *ex vivo* skin gene therapy using an hBPAG2 transgenic mouse strain. In contrast to control mice, DEC-NC16A treated Wt mice showed elongated graft survival. H/E stainings of skin biopsies confirmed this observation, as necrosis or leukocyte infiltration was not detectable in DEC-NC16A treated mice until day 49 post grafting. The mechanism behind this immunosuppressive effect will be examined in further experiments.

P097
Interfering with immune regulation – impact of human plasmacytoid dendritic cells on regulatory T cell mediated suppression

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Malignant melanoma correlates with increased regulatory T cell (Treg) frequencies anticipating anti-tumor T cell responses. Therefore, understanding circumvention of Treg-mediated suppression is mandatory to improve therapies for melanoma patients. Vaccination approaches with monocyte derived dendritic cells (DC) pulsed with tumor antigens display well-proven therapies able to induce transient anti-tumor T cell responses. However, success of treatment is limited and needs further investigation. Studies showed that terminally differentiated DC have the ability to expand Treg thereby augmenting a tolerogenic environment, which impairs anti-tumor immunity. Here we show that both resting and activated plasmacytoid DC (pDC), comparably weak T cell activators, exhibit the capacity to abrogate Treg-mediated suppression. In strong contrast to monocyte derived DC (moDC), pDC do not alter the anergic state of Treg. These pDC properties are independent of proinflammatory cytokines and not due to insufficient activation of Treg. Thus, pDC clearly show tolerance-breaking capabilities without expanding Treg, demonstrating their potential to facilitate anti-tumor responses.

P098

Identification of triptolide as a potential aryl hydrocarbon receptor antagonist in memory T cells

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In China extracts of the herb *Tripterygium wilfordii* Hook F. (TwHF) are successfully used to treat psoriasis and other autoimmune and/or inflammatory diseases due to its favorable cost-benefit ratio. Triptolide has turned out to be the active substance of TwHF-extracts and has been shown to exert potent anti-inflammatory and immune-suppressive effects *in vitro* and *in vivo*.

The immunosuppressive action of triptolide has been generally attributed to suppression of T-lymphocyte activation. Recently, it was found that triptolide inhibited the differentiation of murine CD4+ T cells into Th17 cells and decreased the transcription level of interleukin (IL)-17 mRNA and IL-6-induced phosphorylation of STAT3, a key signaling molecule involved in the development of Th17 cells.

The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor best known for mediating the toxicity of dioxin. It was shown that in a CD4+ T-cell lineage of mice AhR expression is restricted to the Th17 cell subset and its ligation results in the production of the Th17 cytokine IL-22. Ligation of AhR by6-formylindolo[3,2-b]carbazole (FICZ), a tryptophan-derived photoproduct that is thought to be an endogenous agonist with high affinity for the AhR receptor, upregulates the expression of IL-17A, IL-17F and IL-22 in human Th17 cells, as well as induction of genes encoding xenobiotic metabolizing cytochrome P450 enzymes such as CYP1A1 and CYP1B1.

To test whether exposure to triptolide affects differentiation of nave human T cells to effector cells, we added triptolide and FICZ during the *in vitro* differentiation of nave T cells. Comparison of Th17 differentiation in nave T cells by intracellular staining and ELISA after the addition of FICZ together with different concentrations of triptolide showed strongly decreased IFN-γ, IL-17A and IL-22 production in a dose-dependent manner. Realtime-PCR demonstrated a strong down-regulation of IFN-γ, IL-17a and IL-22 mRNA expression by triptolide as well as of CYP1A1 and CYP1B1.

These data suggest that triptolide is capable of altering the AhR transcription pathway and its activity as an AhR-antagonist may be linked to its anti-inflammatory and immune-suppressive effects. Since triptolide is successfully used as a therapeutic of autoimmune disorders in China our identification of triptolide as an AhR-antogonist suggests that the AhR could be a therapeutic target of interest.

P099

Proteolytic activity in bullous pemphigoid is dominated by acidic proteases synthesized by Nc16a binding granulocytes

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It is well known that autoantibodies directed against the major autoantigen of bullous pemphigoid (BP) i.e. Nc16a can readily be detected binding its autoantigen residing in the basement membrane zone. However, it is also known that these autoantibodies are bound by Fc gamma receptors on the cell surface of effector cells as e.g. histiocytes and granulocytes. We therefore used a fluorescence conjugated recombinant Nc16a protein to detect Nc16a binding cells in lesional skin of BP patients. Here, specific binding of this protein could be localized to granulocytes but not to histiocytes near the basement membrane zone, whereas no binding was observed in appropriate controls from pemphigus or lupus biopsies. To further determine the role of these cells in the inflammatory process resulting in blister formation, in situ gelatine zymography under neutral and acidic conditions was performed. Interestingly, in all biopsies investigated ($n = 15$) a prominent gelatinolytic activity colocalizing with the Nc16a binding granulocytes was found under acidic conditions, whereas at neutral pH only 7/15 exhibited a mainly moderate gelatinolysis. Inhibition experiments using class specific inhibitors revealed, that in particular acidic serine proteases and cysteine proteases are the major components of the proteolytic activity in BP biopsies.

These data demonstrate, that in BP Nc16a binding granulocytes induce proteolysis mainly via acidic serine and cysteine proteases.

P100

Monocyte-derived dendritic cells matured with IFN-alpha/TLR-Ligand are more effective in inducing high expression of IFN-gamma in naive CD4+ T cells than dendritic cells generated with a conventional cytokine cocktail

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Despite recent progress in the identification of various subsets of human dendritic cells (DC) in the peripheral blood, monocyte-derived DC are still considered as a valid model for the generation of high numbers of DC *in vitro* and for the use as a means of therapeutic intervention. Conventional *in vitro* generated DC, matured with a cocktail of inflammatory cytokines (IL-1b, TNF-a, IL-6 and PGEB) have been applied in the treatment of cancer patients, though with limited clinical outcome. In our approach, we compared DC, stimulated with the conventional cytokine cocktail, with DC, matured with a mixture of IFN-alpha, IFN-gamma, TNF-alpha, IL-1beta and the TLR3-Ligand poly I:C. Both populations of *in vitro* generated mature DC expressed comparable levels of HLA-DR, maturation markers such as CD83 and molecules of the B7 family, except for B7-H2 which was expressed on a higher percentage, and CD40 as well as ILT-3, which were expressed at a lower density on conventional DC. Yet only IFN/TLR-matured DC secreted detectable amounts of IL-12p70. These DC populations were subsequently used to stimulate allogeneic naive CD4+CD45RA+CD45RO-CD25- T cells and the cytokine profiles (IFN-gamma, IL-4, IL-5, IL-13, IL-9) of the respective T cells were determined in intracellular FACS and ELISA. IFN/TLR-matured DC induced a population of IFN-gamma highly expressing T cells with a Th1 bias, whereas conventional cytokine matured DC generated T cells producing low amounts of IFN-gamma with a cytokine profile rather resembling a Th0 phenotype.

These results may contribute to the refinement of clinical protocols for the *in vitro* generation of effective mature DC for therapeutical intervention in a setting where Th1 responses are beneficial.

P101 (V32)

Human chitotriosidase – a novel cofactor that supports the initial activation of macrophages upon toll-like receptor stimulation

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The skin represents an effective barrier against penetrating pathogens preventing continuous activation of the immune system. However, an impaired skin barrier enables the invasion of pathogens and the induction of inflammation. Initiation of an inflammatory response within the first line of host defence is mediated by pattern recognition receptors (PRR) such as toll-like receptors (TLRs) that facilitate the recognition of pathogen-associated molecules. Various different cell types including macrophages express PRR and respond to pathogen-related molecules by the release of inflammatory cytokines and hydrolytic enzymes such as TNF-α and chitotriosidase (ChT). Although it has been speculated that ChT a chitin degrading enzyme is involved in the defence against chitin-containing pathogens its contribution to the innate immunity is still unknown. Therefore, we have investigated the impact of human ChT on the recognition of specific TLR-ligands and *Staphylococcus aureus* by macrophages. We could show that the specific knock down of ChT leads to a strongly diminished response of macrophages to the specific TLRs ligands LPS and Pam3Cys, crude peptidogylcan preparations and *S. aureus*. Macrophages response was related to the secretion of TNF-α. Moreover, we found that neither the enzymatic activity of ChT nor a direct interaction with the pathogen-related molecules is required for the ChT enhanced inflammatory response. Immunofluorescence staining revealed that ChT is associated with the plasma membrane of macrophages pointing towards a direct contribution to the recognition process. In line with these findings blockage of phagocytosis does not affect the ChT-related enhancement of macrophage activation. In conclusion, we could show that ChT contributes significantly to the recognition of pathogen-related molecules and *S. aureus*. Although the orchestrating molecular mechanism remains to be elusive, ChT appears to play a pivotal role in the initial activation of macrophages via TLRs.

P102

Stable suppression of protein expression via retroviral transduction with shRNA as method to alter T cell function using the example of PD-1 in tumour-specific T cells

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During the last decades our knowledge on the immune system and its regulatory pathways has increased steadily. The activity of immune cells is tightly controlled by positive and negative signals, ensuring immediate action against threats against the organism, while maintaining tolerance against self-peptides. Cancer cells often exploit negative signalling pathways which inhibit T cell function in tumour immune escape, leading to uncontrolled tumour growth. Therefore, cancer immunotherapy which employs the immune system to attack cancer cells has not only to provide a strong immune response but also has to overcome tumour escape strategies. One method which can be used to enhance the tumour directed immune response is RNAi (RNA interference) via siRNA (small interfering RNA), a mechanism by which gene expression can be inhibited sequence-specifically on mRNA level, leading to down-regulated expression of the target protein. SiRNAs can be stably introduced into cells via retroviral transduction with vectors expressing short hairpin RNA (shRNA) which is processed by the cell's enzymatic apparatus into functional siRNA.

We and others have shown previously that PD-L1 (programmed death receptorligand-1) on tumour cells can mediate suppression of tumour-specific T cells via the inhibitory receptor PD-1 (programmed death receptor-1). The transfer of PD-1-/- tumour-specific T cells in an animal tumour model led to improved tumour rejection, and the blockade of PD-L1 with mAbs resulted in enhanced cytokine production by cytotoxic T cells. Therefore, the disruption of this inhibitory pathway could be a promising approach to improve the efficacy of adoptive T cell therapy. To achieve this we established several functional siRNA sequences against PD-1 in murine and human PD-1 expressing cell lines. These sequences were further used to stably reduce PD-1 expression in tumour-specific T cells which led to increased cytotoxic function of T cells in the presence of PD-L1 expressing tumour cells.

Reduction of surface expression of co-inhibitory molecules like PD-1 via RNAi canthus increase T cell immune functions and might become a powerful tool for cancer immunotherapies.

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P103

Expression of IL-1 family members upon stimulation with IL-17 differs in keratinocytes derived from psoriasis patients and healthy donors.

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Background: A number of studies have challenged the T cell centred pathogenetic view of psoriasis by the finding that epithelium-expressed genes are intimately involved in the inflammatory process. IL-17 is an important inflammatory mediator in skin psoriasis.

Objective: IL-17 is known to act on keratinocytes and we were interested in its impact on expression of pro- and anti-inflammatory IL-1 family members.

Methods: We compared human primary keratinocytes derived from psoriasis patients and healthy individuals using qRT-PCR and ELISA.

Results: In the presence of IL-17 psoriasis derived keratinocytes showed a significantly higher induction of the pro-inflammatory members IL-1F6 and IL-1F9 compared with those from healthy individuals but not of anti-inflammatory membersIL-1F5, IL-1F7 or IL-1F3. Both basal, as well as IL-17 induced production of IL-1F2/IL-1β and IL-1F1/IL-1α were found to be significantly lower in psoriasis keratinocytes.

Conclusion: As keratinocytes were derived from epidermal stem cells of the hair follicles and obtained from non-lesional sites, differences found are likely to present an intrinsic feature of psoriasis epithelium. Our data suggest that the significance of IL-1 members as therapeutic targets in psoriasis conditions merits further and thorough investigation.

P104 (V08)

The purinergic receptor P2X7 is a crucial inducer of inflammation in contact hypersensitivity

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Allergic contact dermatitis (ACD) is a T cell mediated inflammatory skin disease and one of the most prevalent occupational diseases. A crucial role for innate immunity that involves triggering of Toll like receptors TLR2 and TLR4 has been shown in the mouse contact hypersensitivity (CHS) model. Since germ-free mice develop CHS, endogenous agonists for pattern recognition receptors (PRR) may trigger

the innate immune response in CHS. We analysed whether extracellular ATP, released from stressed cells as a danger signal is involved the inflammatory response in CHS. ATP triggers the purinergic receptors such as P2X7. This receptor activates the NLRP3inflammasome that processes pro-IL-1β and pro-IL-18 via caspase-1.
We investigated the role of the ATP receptor P2X7 in CHS, using C57BL/6 or P2X7-/-mice. In addition, we investigated the involvement of the NLRP3 inflammasome in the ATP response using ASC-/- and NLRP3-/- mice. CHS responses were determined by ear swelling. In addition, ATP production in situ and IL-1β processing in dendritic cells (DC) as well as T cell responses *ex vivo* were analysed.
Here we demonstrate that P2X7-deficient mice are resistant to CHS. Moreover, P2X7-/- DC lack the ability to sensitize both wild type and P2X7-deficient recipients against the contact sensitizer TNCB. Suppression of P2X7 signaling by the antagonists suramin or KN-62, or removal of extracellular ATP by the ATP degrading enzyme apyrase can prevent CHS. *In vivo* bioluminescence imaging revealed that treatment of mice with contact sensitizers induces ATP release from skin cells.
LPS primed P2X7-deficient BMDCs did not release mature IL-1β in response to ATP treatment. Pretreatment with the P2X7-independent inflammasome activator alum restored the sensitizing potential in CHS and the ability to release IL-1β *in vitro*. Blocking IL-1 signaling *in vivo* by using the IL-1 receptor antagonist Anakinra also prevented CHS.
These findings clearly show that contact allergens such as TNCB and oxazolone indirectly activate PRR signaling via endogenous agonists. Up to now, ACD treatment only comprises symptomatic therapy using immunosuppressive drugs such as corticosteroids. Inhibition of P2X7 signaling is an important step towards new causative treatments by specific innate immune modulation.

P105 (V16)
Spontaneous inflammatory blistering in a new passive transfer model of bullous pemphigoid in adult mice

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Bullous pemphigoid (BP) is an autoimmune blistering skin disease associated with autoantibodies mainly recognizing the hemidesmosomal proteins BP180/collagenXVII and BP230 of the dermo-epidermal junction (DEJ). In patients skin deposition of IgG and complement C3 at the DEJ and infiltration with granulocytes in the upper dermis are observed. Binding of IgG autoantibodies to the DEJ, which triggers local complement activation and the recruitment of inflammatory cells, are prerequisites for dermal-epidermal separation in experimental pemphigoid models. Passive autoantibody transfer models of pemphigoid have been established in wild type and collagen XVII-humanized mice. While recapitulating the main features of the human disease, the skin blistering does not develop spontaneously in these models. In addition, due to their experimental design they allow only short-term observation of diseased animals. Previously, we observed that the passive transfer of rabbit IgG against collagen XVII into adult mice induces spontaneous skin blisters, which are however limited to the injection sites and associated with very low complement deposition and granulocyte recruitment. It is known from models of experimental glomerulonephritis, that the passive transfer of rabbit or sheep IgG against the glomerular basement membrane induces extensive inflammatory disease in mice immunized with corresponding heterologous IgG. Therefore, to reproduce more faithfully the human disease, we established an animal model in adult mice characterized by inflammatory spontaneous blistering and allowing for longer observation times. For this purpose, we immunized adult SJL-1, BALB/c and C57BL/6 mice ($n = 20$) with rabbit IgG and subsequently transferred rabbit IgG against murine collagen XVII every second day for 14 days. After the intraperitoneal injection of collagen-specific antibodies, mice were examined over a period of 20 days. The lesions, including blisters, erythema, and erosions with crusts developed at distant predilection sites such as ears, snouts and limbs. The mice showed extensive spontaneous blistering, which correlated with the levels of circulating pathogenic rabbit IgG measured by ELISA. Histopathological analysis of lesional skin revealed dermal-epidermal separation and an inflammatory infiltrate dominated by granulocytes. By direct immunofluorescence microscopy, deposits of rabbit IgG and murine complement C3 at the DEJ were found in the perilesional skin. In conclusion, our study highlights the key features of a new mouse model for BP and should prove valuable in dissecting the mechanisms of blister formation and in developing new therapeutic strategies for pemphigoid diseases.

P106
Combined treatment with immunoadsorption and rituximab leads to fast and prolonged clinical remissions in refractory pemphigus.

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Context: Pemphigus is a potentially fatal blistering autoimmune disease affecting the skin and mucous membranes. The standard therapy consists of high-dose systemic corticosteroids in combination with immunosuppressive agents often causing severe comorbidity. Recently therapeutic strategies such as immunoadsorption and theanti-CD20-antibody rituximab aim at a more specific inhibition of pathogenic autoantibodies in pemphigus.
Objective: The aim of this study was to investigate the long-term efficacy of adjuvant immunoadsorption in combination with rituximab in pemphigus patients refractory to high-dose immunosuppressive therapy.
Design: We retrospectively analyzed the clinical and serological parameters of six patients with mucocutaneous and four patients with mucosal pemphigus vulgaris. Inclusion criteria consisted of acute pemphigus and refractory disease was defined as previous treatment with high-dose glucocorticoids and adjuvant immunosuppressives for at least 3 months. None of the patients received second-line therapies such as immunoadsorption, rituximab or intravenous immunoglobulins 6 months before entering the present study. All 10 patients were treated with two to four cycles of adjuvant immunoadsorption at 4 weeks intervals, each cycle consisting of 4 consecutive treatment days, depending on the clinical response. Following IA, rituximab was given either at a dose of 1000 mg twice on days 1 and 15 or at a dose of 375 mg/m/BSA four times in weekly intervals, in most of the patients. The follow-up period was 12 months for seven patients and three patients were observed over 6 months. During the follow – up the autoimmune bullous skin intensity score (ABSIS) was applied to assess the clinical response, anti-desmoglein 3 (Dsg 3)/ - Dsg 1autoantibody titers were controlled monthly and systemic corticosteroids were tapered according to the individual clinical status.
Results: At baseline nine of 10 patients demonstrated extensive oral involvement (ABSIS mucosa: 6.5 5.5 points; median range) followed by a rapid and statistically significant response 1 month after treatment (ABSIS mucosa: 2.0 2.0 points).Moreover, this remission of oral lesions persisted up to 12 months after therapy. Correspondingly, immunoadsorption and rituximab treatment resulted in a decrease of anti-dsg3-IgG levels to 48.5% 66% of the initial values after 1 month and to 16% 57% after 12 months. Six patients with mucocutaneous pemphigus showed extensive cutaneous blistering at baseline (ABSIS skin: 16.5 12.1 points). Upon follow-up there was a significant decrease in ABSIS skin score 1 month after therapy (5 12 points) and, except for one patient suffering from a relapse, five patients remained in clinical remission 12 months after treatment (ABSIS skin: 1 3 points). According to the clinical condition, anti-Dsg1-IgG concentrations decreased to 50% 44% of the initial values after 1 month and to 13% 24% of the initial value after 12 months. Appropriately, systemic corticosteroids were tapered in all cases and prednisolone doses were significantly lower at 12 months follow-up compared with the beginning of the study. Both immunoadsorption and rituximab treatment were well tolerated without any severe adverse events during the follow-up period.
Conclusions: The present findings suggest that the combination of immunoadsorption and rituximab induces both a rapid clinical remission and leads to a long-term control of disease activity in severe refractory pemphigus patients.

P107
Impact of keratinocyte-derived type III interferon (IFN λ) in cutaneous lupus erythematosus and related autoimmune disorders

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Background: Type I interferons (IFN) have been shown to play a central role in the pathophysiology of lupus erythematosus (LE). The recently discovered type III IFNs (IFNλ) share several functional similarities with type I IFNs. Since IFN lambda has been shown to act primarily on epithelial cells, we investigated the function of type III IFNs in the proinflammatory network in cutaneous LE (CLE) and related disorders.
Patients and Methods: The ability of keratinocytes to produce IFN lambda in response to immunostimulatory nuclear acids was determined in cell culture experiments using epidermal explants and primary human keratinocytes. IFN lambda expression in skin biopsies and IFN lambda serum levels were measured in patients with cutaneous LE, dermatomyositis (DM), lichen planus (LP) and healthy controls by immunohistochemistry and ELISA. The functional impact of IFN lambda on lymphocyte recruitment was proven in cell migration studies.
Results: IFN lambda1 and the IFN lambda-receptor are strongly expressed in active skin lesions of patients with CLE, LP and DM. IFN lambda1-serum levels are significantly enhanced in CLE patients and this increase is associated with the extent of skin lesions. Functional analyses revealed that human keratinocytes are able to produce high levels of IFN lambda1 but only low amounts of IFN alpha/beta/gamma in response to poly IC, suggesting that IFN lambda is a major IFN produced by these cells. IFN lambda1-stimulation induces the expression of several proinflammatory cytokines in keratinocytes, including CXCL9, which drive the recruitment of immune cells and are associated with the formation of CLE skin lesions.
Conclusion: Our observations provide several lines of evidence that keratinocyte-derived IFN lambda has a role in the pathophysiology of CLE. First, IFN lambda was strongly expressed in the epidermis of CLE skin lesions. Second, enhanced IFN lambda levels could be measured in the serum of patients with active disease. Third, the lesional expression pattern of IFN lambda correlates with that of the IFN lambda-inducible chemokine CXCL9, compatible with the notion that IFN lambda drives the inflammatory recruitment of immune cells. Based on our *in vitro* studies with explanted epidermal sheets, cultured keratinocytes and cell migration analyses, it is highly plausible that the IFN lambda1 in CLE patients derives from keratinocytes and supports the formation of inflammatory skin lesions. Our results provide first evidence for a role of type III IFNs not only in anti-viral immunity but also in autoimmune diseases of the skin.

P108
Poly IC induces IFN lambda expression in keratinocytes via endosomal (TLR-dependent) and cytosolic (TLR-independent) pattern recognition receptors

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Background: The synthetic immunostimulatory nucleic acid poly IC is a strong inducer of type III interferon production (IFN lambda) in keratinocytes. Interestingly this stimulation, as well as infection of keratinocytes with vesicular stomatitis virus, has an only minor effect on the levels of other interferons (alpha, beta, gamma), suggesting that IFN lambda is the major interferon of these cells. Poly IC has been found to have the capacity to stimulate innate immune responses via endosomal TLR-dependent (TLR3) and cytosolic (MDA5) pattern recognition receptors (PRR), but the mode of action in keratinocytes has remained unclear.
Methods: Human and murine keratinocyte cell lines, including MDA5-knockout cells, were cultured and stimulated with different synthetic ligands of endosomal and cytosolic PRRs. Additionally, chloroquine and inhibitory IPS1 siRNA were used to disable endosomal and cytosolic PRR pathways. RT-PCR and ELISA were used as readout systems.
Results: Chloroquine reduces the poly IC-induced IFN lambda expression by half, the same effect was seen using inhibitory siRNA for IPS1. The combination of both (chloroquine + IPS1 siRNA) totally avoided IFN lambda induction. Similar results were found for the proinflammatory cytokines including CXCL9 and IL6.
Conclusion: Poly IC induces the expression of type III interferons via TLR-dependent and cytosolic pattern recognition pathways. Our results demonstrate that different innate immune response pathways drive the induction of keratinocytic IFN lambda expression and support the view that the type III IFN system has an outstanding role for the innate immune response of these cells.

P109
The Molecular Profile of Psoriatic Skin in Responders to Ustekinumab or Etanercept Following Twelve Weeks of Treatment: Results from the ACCEPT Trial

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Question: To assess the impact of p40 cytokine (IL-12/IL-23) or TNF-alpha blockade on resident and inflammatory cells and on the expression of gene circuits that may drive chronic immune activation and inflammation in the skin.
Methods: In ACCEPT, a randomized, active-controlled study, the efficacy of etanercept and ustekinumab were compared in 903 patients with moderate-to-severe plaque psoriasis through wk 12. Skin biopsies were performed in a subset of patients at baseline, wks 1 and 12. Microarray analyses (Affymetrix U133+2 array) comparing non-lesional skin ($n = 85$) to lesional skin ($n = 85$) at baseline showed several thousand probe sets differentially expressed (>2-fold change FDR, $P < 0.05$) in lesional skin.
Results: Patients responding to each agent (PASI75, $n = 21$ for etanercept, $n = 19$ ustekinumab) had significant changes in approximately 4000 transcripts compared to untreated lesions, indicating significant resolution of pathological gene circuits. A set of 2922 transcripts, which included S100 genes, keratins 6/16, and innate defense products (cytokine-modulated genes in keratinocytes), were commonly regulated by ustekinumab or etanercept. The top ten genes down-regulated at wk 12 by ustekinumab overlap with nine of the top 10 genes down-regulated by etanercept at wk 12; only two of the top 10 genes up-regulated overlap (NTRK2, THRSP) in this comparison. The genes up-regulated by ustekinumab include a number of keratin structural proteins indicating a unique effect of ustekinumab on keratinocytes.
Conclusion: Elucidation of common and unique effects of ustekinumab and etanercept define critical pathways involved in psoriasis pathogenesis and a successful therapeutic response. Broad genomic

assessments provide an independent way to judge the extent to which disease pathology can be reversed by effective therapeutics.

P110
TNF-alpha mediates a delayed anti-inflammatory feedback mechanism in the response of monocytes to LPS

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Lipopolysaccharide (LPS) is the major component of the outer membrane of Gram-negative bacteria. Recognition of LPS by monocytes initiates a rapid release of inflammatory mediators resulting in activation of leukocytes. However, a reliable analysis of the delayed response of monocytes elicited by LPS is not yet done.

Using oligonucleotide microarrays we performed a sophisticated genome-wide analysis to define the delayed LPS-triggered expression profile in monocytes after 16 h. Blocking individual protein kinases known to be involved in LPS-dependent activation of monocytes demonstrated that LPS-modulated transcripts are mainly regulated in a MAP-kinase p38-dependent manner. Interestingly, statistical analysis demonstrated that LPS induced an up-regulation of anti-inflammatory rather than pro-inflammatory molecules at this delayed time point.

Indeed, monocytes challenged for 16 h with LPS were able to inhibit the LPS-induced expression of pro-inflammatory cytokines like TNF-alpha, CXCL-9, CXCL-10 and CXCL-11 from macrophages in co-culture experiments. Thus, prolonged LPS treatment resulted in the generation of an anti-inflammatory monocyte phenotype which actively suppresses pro-inflammatory responses from other innate immune cells.

Interestingly, many genes expressed by these anti-inflammatory monocytes were known to be regulated by pro-inflammatory TNF-alpha. Thus, we blocked TNF-alpha signalling during generation of anti-inflammatory monocytes using soluble TNF-receptors. When these monocytes were subsequently was hed and transferred to macrophages we observed a significant reduction of their capacity to inhibit the release of pro-inflammatory mediators by macrophages after LPS-stimulation.

Therefore, our data indicate an important effect of TNF-alpha in the generation of an anti-inflammatory monocyte phenotype during the late phase of monocyte activation by LPS. This may explain the unexpected deleterious effect of TNF-blocking therapeutics in the treatment of sepsis. Improved understanding of this anti-inflammatory feedback mechanism in the response to pathogenic microbes is important for the development of new therapeutic regimes for infections as well as inflammatory disorders.

P111
Cytokine-induced cell cycle arrest in isolated cancer cells

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Even though most established tumor immunotherapies are based on tumor cell destruction by cytotoxic cells, an increasing number of data shows that successful cancer immunotherapy depends on interferon-γ (IFN-γ) producing T cells, i.e. T helper 1 (Th1) cells. RIP1-Tag2 mice undergo multistage carcinogenesis by expressing the oncoprotein T antigen 2 (Tag2) of the Simian Virus 40 under control of the rat insulin promoter (RIP) in all beta (β) cells (islet cells) of the pancreas. We previously showed, that Tag-specific Th1 cells double the lifespan of RIP1-Tag2 mice by decreasing the proliferation rate of tumor cells and by inhibiting tumor angiogenesis without causing either tissue destruction or apoptosis *in vivo*. The therapeutic effect of the Tag-specific Th1 cells were critically dependent on IFN-γ and TNF signalling.

To unravel the underlying mechanisms, we investigated the direct effects of the two key cytokines, IFN-γ and TNF, on isolated cancer cells from RIP1-Tag2 mice. Therefore, we analysed the cancer cell cycle by Brdu/7-AAD double-staining and flow cytometry, and PCR arrays concerning cell cycle genes. Additionally, we determined the apoptosis rate by caspase 3/7 activity assay and subG1 analysis and assessed proliferation by BrdU-proliferation-assay and Ki67-staining. To specify the signalling pathways, we further examined cancer cells from RIP1-Tag2×TNFR1-/- (TNF pathway) and RIP1-Tag2×–STAT1-/- (IFN-γ pathway) using the same assays as described above.

We found a significant suppression of the proliferation rate of the isolated RIP1-Tag2 tumor cells *in vitro* by IFN-γ and TNF, accompanied by a cell cycle arrest in G1. On the other hand, none of the two cytokines caused detectable apoptosis-induction (no increase of subG1 cells and caspase 3/7 activity). The effects of IFN-γ and TNF were specific and strictly required both STAT1 and TNFR1. IFN-γ failed to induce cell cycle arrest in STAT1-deficient cancer cells, and TNF failed to arrest cell cycle in TNFR1-deficient cancer cells. Using PCR arrays we found that IFN-γ strongly affects the expression of cell cycle genes specifically regulating G1/G0 arrest.

Taken together, our data suggest that Tag-Th1 mediated immunotherapy causes IFN-γ- and TNF-dependent cell cycle arrest in the absence of cancer cell destruction.

P112 (V34)
Both loss of tolerance to type VII collagen and autoantibody-induced tissue injury are genetically controlled in experimental EBA

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Epidermolysis bullosa acquisita (EBA) is an autoimmune blistering disease, characterized by antibodies to type VII collagen (COL7). EBA can be induced in mice either by transfer of anti-murine COL7 IgG or by immunization with a fragment of murine COL7. In contrast to other autoimmune diseases, e.g. rheumatoid arthritis, little is known about the genetic susceptibility for EBA. We therefore used two EBA mouse models to address the hypothesis that both disease induction (immunization-induced) and autoantibody-induced tissue injury (IgG transfer) are genetically controlled in these experimental models. Mice from 10 different inbred strains, as well as more than 250 mice from an autoimmunity-intercross line, involving four different strains, were immunized with recombinant murine COL7. Induction of EBA in inbred strains was almost exclusively restricted to mice with the MHC haplotype H2s. In addition, 33% of mice from the autoimmunity-prone intercross line developed EBA skin lesions after immunization. In these latter mice, susceptibility to EBA was controlled by 6 loci outside the MHC, located on chromosomes 1, 6, 9, 12 and 19. Likewise, autoantibody-induced tissue injury in the autoantibody transfer model of EBA also showed variability among 15 inbred mouse lines. Specifically, C57Bl/6 mice were highly susceptible to EBA induction by antibody transfer, while several other strains, including NOD or FVB mice, were completely protected. We then used publically available genotyping data from these inbred mouse lines to identify gene loci associated with autoantibody-induced tissue injury. Indeed, this analysis identified several loci controlling autoantibody-induced tissue damage. In summary, our findings show that both, loss of tolerance and autoantibody-induced tissue injury are genetically controlled in experimental EBA. The identified genes provide further insight into the pathogenesis of this disease which may ultimately facilitate the development of novel treatment strategies.

P113
Mast cells in psoriatic lesions express CD137 receptor

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CD137, a member of the TNF-α superfamily, is known for its T cell co-stimulatory capacity. Stimulation of 4-1BB, the homolog of CD137 in murine mast cells, induces secretion of IL-6 and TNF-α, two key players in the immunopathology of psoriasis. Therefore, we asked whether CD137 receptor is expressed in mast cells of psoriatic skin. We investigated skin samples from psoriasis patients and from normal donors (skin obtained by breast reduction surgery), using both conventional light microscopy and dual-color confocal fluorescence microscopy. We found a clear increase in mast cell numbers in psoriatic plaques compared to normal skin. In normal skin, the expression of the CD137 receptor was not detectable. In contrast, a significant expression of CD137 receptor was found in cell infiltrates of psoriatic lesions. To further characterize the cells that express CD137 receptor in lesional skin, we performed double immunofluorescence staining using specific antibodies against CD137 receptor and tryptase. We found that most of the cells that stained positive for CD137 receptor also stained positive for tryptase indicating these to be mast cells. Our data suggest that mast cells are not only present in increased numbers in psoriatic lesions but are also activated, as indicated by the expression of the CD137 receptor. Therefore mast cells activated via CD137 receptor may be an important source for secretion of proinflammatory cytokines such as IL-6 and TNF-α, especially in the early phase of development of psoriatic plaques.

P114
Effect of Bisphenol A on dendritic cell maturation and T cell plasticity

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Bisphenol (BPA) is a main xenoestrogen commonly used in the plastic industry. It has been shown that maternal exposure with BPA promotes the development of experimental asthma in mouse pups and that BPA affects the differentiation of nave T cells (TC). In the present study we investigated the effect of low dose BPA on dendritic cell maturation and TC plasticity.

The effect of BPA on the immune function of monocyte-derived dendritic cells (MoDC) and nave CD4+ TC was analyzed by flow cytometry. Low dose treatment with BPA did not influence the expression of differentiation and maturation marker on MoDC while the expression of the homing receptor CD62L on nave CD4+ TC was slightly reduced.

These results demonstrate that BPA does not influence MoDC maturation, however, it may influence TC homing properties.

P115
Psoriatic cytokines induce insulin resistance in T-lymphocytes

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T-lymphocytes play a central role in immune mediated diseases like psoriasis. In this chronic inflammatory skin disorder adhesion molecule mediated rolling on the endothelium is a prerequisite for the extravasation of lymphocytes into the skin, where they contribute to the phenotypic aspect of the disease. Activated T-cells and keratinocytes produce pro-inflammatory cytokines, which also have systemic effects. Thus psoriasis has been associated with comorbidities such as hypertension, atherosclerosis, and insulin resistance often leading to the manifestation of type 2 diabetes mellitus.

The molecular mechanisms of insulin resistance have been intensively examined in classical insulin-responsive tissues (muscle, fat, liver). Whether insulin resistance also occurs in immune cells and which causal role pro-inflammatory cytokines might play in this context has not yet been sufficiently investigated. We hypothesize that insulin resistance of inflammatory T-cells represents a pathomechanism during the development of the disease.

We could show that primary T-lymphocytes as well as a T-cell line become resistant to insulin stimulation by cytokines that are typical for the psoriatic inflammation such as TNF-α, IL-17 and IL-23. In addition insulin signalling influences the expression of adhesion molecules which are dysregulated by inflammatory stimuli contributing to the pathological attachment of lymphocytes to the endothelium. Therefore we suggest that inflammation induced insulin resistance has not only metabolic consequences for psoriatic patients, but also represents a mechanism by which extravasation of lymphocytes is mediated.

P116
Fumarate-induced HO-1 differentially regulates the expression of IL-23 and IL-12

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Data from clinical studies have shown that fumarates improve multiple sclerosis and psoriasis, two diseases in which both IL-12 and IL-23 are responsible for pathogenic T helper (Th) cell differentiation. However, both diseases show opposing responses to most of the established therapies. Previously, we have shown that fumarate-treatment in humans induces IL-4-producing Th2 cells *in vivo* and generates type II dendritic cells (DC) that produce IL-10 instead of IL-12 and IL-23. Here we show that in mice fumarates also generate type II DC that promoteIL-4-producing Th2 cells and suppress Th1 and Th17 cells *in vitro* and *in vivo*. Importantly, fumarates protect mice from experimental autoimmune encephalomyelitis (EAE). Anti-inflammatory type II DC result from fumarate-induced glutathione-depletion and induction of reactive oxygen species (ROS). Interestingly, fumarates induced the transcription of the ROS sensitive heat shock protein HO-1, which is implicated in the regulation of inflammatory immune responses. To further analyze the mechanism of fumarate-induced immune modulation we studied the interaction between fumarates on HO-1, IL-12 and IL-23 expression during immune activation. Fumarate treatment induced HO-1 in DC *in vitro* and *in vivo*. This was associated with a decrease in IL-12/IL-23p40 and IL-23p19. As HO-1 is aROS-sensitive heat shock protein, we determined the direct impact of HO-1 induction on either IL-12 or IL-23 production. To address this point we transfected DC with HO-1 siRNA prior to fumarate treatment and TLR4 stimulation. As expected, HO-1siRNA prevented LPS-mediated induction of HO-1. Importantly, HO-1 siRNA fully restored IL-23p19 expression in fumarate-treated DC. Moreover, it partly restored IL-12/IL-23p40 mRNA expression and as a consequence IL-23 protein production. In sharp contrast, HO-1-siRNA had no effect on the expression of IL-12p35 mRNA. Since HO-1 has been reported to interact with AP-1 and NF kappa B proteins, both transcription factors involved in IL-23 expression, we further characterized if HO-1 interferes with the promoter activity of IL-23. By chromatin immunoprecipitation analysis we could show that HO-1 binds to AP-1 and c-Rel binding sites of the IL-23 promoter in fumarate-treated DC but not control DC after TLR4 activation. This was associated with epigenetic modifications of the IL-23 promoter locus as shown by decreased histone 3 acetylation after fumarate-treatment. In conclusion, fumarate-induced HO-1 specifically inhibits IL-23 expression by binding to its promoter without affecting IL-12p35.

P117

Connecting expression of the immunological cell stress indicator ULBP2 to the tumor suppressor activity of p53

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The activating immunoreceptor NKG2D, expressed on Natural Killer (NK) cells and different subsets of T cells, and its ligands MHC class I chain-related (MIC) and UL16 binding protein (ULBP) molecules, play a key role in tumor immunosurveillance. A variety of malignancies show surface expression of MIC and ULBP molecules, sensitizing them for NK cell- and T cell-mediated cytotoxicity. However, tumors can subvert NKG2D-mediated immunosurveillance by ligand shedding. In sera from melanoma patients we detected increased levels of both NKG2DL. Interestingly, elevated soluble ULBP2, in contrast to soluble MICA, significantly correlates with poor patient prognosis indicating that differences between both ligands exist with respect to expression and clinical significance. Based on these findings we set out to define signals that control ULBP2 expression in melanoma. We observed that treatment of the tumor cells with different chemotherapeutics, like cisplatin and doxorubicin, strongly upregulates the surface expression of only ULBP2 on melanoma cells. ULBP2 induction was detectable also at the levels of total cellular protein and specific mRNA. Blockade of ATM kinase activity by the specific inhibitor KU-55933 abrogated ULBP2 induction, pointing to an involvement of the DNA damage signalling pathway in ULBP2 regulation. To test whether ULBP2 induction is p53 dependent, we analyzed the ULBP2 mRNA and protein levels in HCT 116 wt and HCT116 p53-/- cells upon cisplatin treatment. By comparison of both cell lines we observed a stronger induction of ULBP2 mRNA and protein levels in HCT116 wt cells, indicating that p53 plays a role in ULBP2 regulation. However, also p53-independent mechanisms affect ULBP2 expression, since cisplatin increased ULBP2 mRNA and protein levels in HCT 116 p53-/-, albeit to a lesser extent when compared to HCT 116 wt. In summary, our data demonstrate a strong clinical significance of ULBP2 expression in melanoma and point to an involvement of p53 in ULBP2 regulation.

P118

Curcumin protects from autoimmune disease by modulating DC differentiation and suppressing Th1 and Th17 cells

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Curcumin (diferuloylmethane) is a naturally occurring yellow pigment isolated from the rhizomes of the plant Curcuma longa. Curcumin has been reported to have immune-modulatory activities and therefore is traditionally used in inflammatory disorders in some regions of Asia. However, the underlying mechanisms that could explain its anti-inflammatory activity are unclear. Here, we studied the effects of curcumin on immune responses *in vitro* and *in vivo*.

First, we studied the effects of curcumin on dendritic cell (DC) differentiation and maturation. DC were treated with concentrations of curcumin that did not affect cell survival or phenotype. Treatment of DC with curcumin resulted in a dose-dependent inhibition of the production of IL-12 and IL-23 after activation through TLR4. In contrast, IL-6 or IL-10 expression was not affected. Analysis of multiple signaling cascades in DC showed that curcumin inhibits TLR4-stimulated NF kappa B and JNK activation whereas p38 phosphorylation was enhanced. Since p38 activation is associated with oxidative stress, we further studied the role of curcumin on the redox system in DC. Curcumin-treated DC showed increased levels of reactive oxygen species, subsequently increased levels of glutathione. Pretreatment of DC with antioxidants restored the TLR4-induced IL-12 and IL-23 production observed in Curcumin-treated DC. *In vitro*, such curcumin-primed DC induced IL-4-producing Th2 cells while inhibiting IFN-gamma and IL-17 production in CD4+ T cells. GATA3 and IL-4 induction was not observed when T cells were activated by anti-CD3/CD28 and treated with curcumin in the absence of DC. Next, we studied the effects of curcumin *in vivo*. Mice were fed curcumin during immunization for experimental allergic encephalomyelitis (EAE). Three to seven days after immunization lymph nodes were excised and analyzed for the expression of transcription factors and cytokines. While the expression of IL-12 and IL-23 as well as T-bet and ROR gamma t was inhibited in curcumin-treated mice compared to control-treated mice, GATA3 expression was induced. This was associated with an inhibition of Th1 and Th17 cells and an induction of Th2 cells. Neither antigen-dependent proliferation nor FoxP3 expression in T cells were affected by curcumin treatment. Importantly, curcumin-fed mice developed no or only mild clinical signs of EAE compared to control-fed mice. Similarly to the situation *in vitro*, also *in vivo* the administration of antioxidants was able to diminish the anti-inflammatory and protective role of curcumin as shown during EAE. Thus, Curcumin-treatment seems to be a promising therapeutic approach for autoimmune diseases by using natural extracts with immuno-modulating properties.

P119

The CD18 hypomorphic psoriasis mouse model – insight into the pathogenesis of a complex disease

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Psoriasis is a chronic disease affecting skin in 2–3% of the general population. We previously showed that the CD18hypo PL/J mouse with a mutation resulting in a reduced expression of the common chain of $\beta 2$ integrins (CD11/CD18) spontaneously develops a skin disease that closely resembles human psoriasis. Interestingly, when backcrossed onto the C57BL/6J background no psoriasiform dermatitis developed, suggesting that apart from the CD18 hypomorphic mutation a small number of modifier genes are required for the precipitation of the disease. Backcross analysis between susceptible CD18hypo PL/J mice and the resistant CD18hypo C57BL/6J strain and a genome-wide linkage analysis identified susceptible loci on chromosome 6 and chromosome 10. Using a congenic approach, we identified a 9-cM fragment on chromosome 10 with genes being responsible for the psoriasiform disease. As we recently found that regulatory T cells as well as activated macrophages play a central role in the psoriasiform disease, our efforts will now concentrate on identifying potential modifier genes which in conjunction with theCD18 hypomorphic mutation are responsible for the dysregulation of these cellular key players and the psoriasiform disease.

P120

Staphylococcus aureus adherence to human endothelial cells depends on von Willebrand factor and shear flow

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Staphylococcus aureus is a frequent facultative human pathogen. Persistent nasal carriage is present in 2–30% of the human population and colonization of the skin is found mainly, at intertriginous areas. By contrast, the skin of 80–100% of patients with atopic dermatitis is colonized with *S. aureus* and *S. aureus* infection is the most common complication of atopic dermatitis. A high risk factor in systemic infections such as endocarditis or sepsis is the overcoming of the skin barrier by bacteria in case of severe skin lesions. The adhesion of *S. aureus* on the endothelium is regarded as the pivotal step in the pathogenesis of such endovascular infections. *S. aureus* expresses numerous surface structures to adhere to the endothelium, e.g. surface-associated adhesins. Activated endothelial cells secrete von Willebrand factor (VWF), which is recognized by the adhesin staphylococcal protein A (SpA). Therefore we studied the interaction between SpA with VWF secreted by the endothelium upon stimulation. To mimic the *in vivo* environment we performed experiments under laminar flow conditions using two different microfluidic devices. Human umbilical vein endothelial cells (HUVECs) were perfused with fluorescence-labelled, fixed *S. aureus* (strain Cowan I) enabling quantification of bacterial adhesion by fluorescence microscopy. To investigate the role of SpA we compared the adhesion of *S. aureus* wild type to the non-pathogenic *Staphylococcus carnosus* and to a SpA-deficient isogenic mutant. We could show that *S. aureus* adherence to endothelial cells was mainly mediated by VWF. Previously, we showed (S. W. Schneider et al. PNAS 2007 104 (19) 7899–7903) that VWF is a shear activated glycoprotein, increasing its binding properties at high shear rates. In line with these data we could show that *S. aureus* adhesion is shear flow-dependent as it increases at higher shear rates (range from 1 up to 50 dyne/cm). At low shear rates SpA plays a crucial role for bacterial adhesion. Moreover we could demonstrate that higher shear rates enables SpA-independent binding of *S. aureus* to VWF by using a SpA-deficient mutant. In conclusion, our study demonstrates that shear flow significantly affects the adhesion of *S. aureus* to the endothelium via VWF. At low shear rates SpA-mediated adhesion of *S. aureus* plays a pivotal role during vascular infections. However *S. aureus* adhesion to VWF can be mediated by an additional molecular mechanism. Therefore we could speculate that increasing shear flow supports *S. aureus* adherence and represents a risk factor for intravascular *S. aureus* infection.

P121

ATF3 causes susceptibility to opportunistic infections during post-septic immunosuppression

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Post septic immunosuppression, also known as compensatory anti-inflammatory response syndrome (CARS), causes most sepsis-related deaths. Yet, the molecular mechanisms underlying this phenomenon are elusive as it paralysis all immune functions. Analysing blood samples of humans during CARS, we found a significant and close correlation of severely suppressed glutathione-levels with the strong induction of ATF3 (activating transcription factor 3) and the loss of activation induced IL-6. ATF3 is the first transcription factor in the NF-κB signalling pathway induced after innate immune stimulation. Thereby, ATF3 negatively regulates the transcription of IL-6 and TNF. As IL-6 and TNF are key cytokines involved in the antimicrobial defence, we speculated that ATF3 might be a key transcription factor responsible for the post septic immune suppression and the increased susceptibility to opportunistic infections. 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 wild type (wt) and ATF3-/- mice, to closely imitate the clinical conditions in mice. Subsequently, we challenged mice during the post septic CARS with the fungal pathogen *Aspergillus fumigatus*, at doses that are non-pathogenic to healthy mice. Post-septic wt-mice rapidly succumbed to this sub-lethal pulmonary *Aspergillus fumigatus* infection. In sharp contrast, ATF3-/- mice had not only a significantly prolonged survival, 20% of these mice even survived this infection that was lethal in100% of wt mice. Thus, ATF3 is the first transcription factor identified that determines susceptibility to and the course of opportunistic infections.

P122

ROS-induced ATF3 protects against bacterial toxins but causes IL 6-dependentsusceptibility to bacterial and fungal sepsis

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One hallmark of sepsis is the generation of oxidative stress and a rapid increase in reactive oxygen species (ROS). Activation of the stress-sensitive negative transcription factor ATF3 provides protection against endotoxin-induced death by a negative feedback loop in the NFκB signal pathway. We recently found that ROS stress, as it occurs during sepsis, strongly enhances ATF3 expression and production by LPS-triggered dendritic cells or peritoneal macrophages. *In vivo*, ROS stress results in glutathione depletion and enhances LPS-induced ATF3 4-fold. Thus, ATF3 inhibited IL-6 mRNA and protein production >90% *in vivo*, and significantly decreased the risk of LPS-induced lethality. This protection was fully reversed by the ROS scavenger N-acetyl-cysteine and strictly dependent on ATF3-induction, as ROS stress affected neither cytokine production nor survival in ATF3-/- mice.

We speculated that the increased awareness of ATF3-/- mice to innate signals such as LPS established solid protection against systemic infection. To test this hypothesis we investigated bacterial infection after cecal perforation (CLP) that were lethal in100% of wild type (wt) mice. At identical conditions of CLP >90% of ATF3-/- mice survived this bacterial peritonitis and reduced bacterial load by 100-fold. Depletion of gluthatione further increased susceptibility of wt mice to CLP, whereas ATF3-/- remained unaffected. In contrast to ATF3-/- mice, all ATF3-/-×IL-6-/- double knock out mice died from CLP directly showing that ATF3-mediated suppression of IL-6 caused susceptibility to bacteriemia. These insights are essential for the management of bacterial and fungal infections, especially for the increasing community of immunocompromised patients.

P123

Co-factor dependent anaphylaxis driven by innate immune signals is mediated by basophils

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Type I allergic reactions are induced by binding of allergens to IgE/FcεRI complexes on mast cell and basophil surfaces. FcεRI cross linking initiates signal transduction and the release of preformed mediators like histamine and platelet activating factor (PAF), eliciting the clinical symptoms. It is well known that some forms of anaphylaxis depend on co- or augmentation factors as best documented for wheat dependent exercise induced anaphylaxis. Other well documented co-factors can be alcohol consumption or infections. However, how infections trigger anaphylaxis is still enigmatic, in parts, because no *in vivo* model for infection dependent anaphylaxis existed. To analyze how innate immune signals may augment mast cell or basophil reactivity and systemic anaphylaxis, we established a new mouse model: Mice were actively sensitized with Ovalbumine (OVA) and challenged with titrated doses of OVA to determine the threshold dose of OVA eliciting systemic anaphylaxis. Anaphylaxis was measured by detecting core body temperature decrease, decrease in blood pressure, and systemic levels of histamine. Challenge with the OVA dose just below threshold resulted in a weak response (temperature drop of -1, 920, 26°C). In contrast, pretreatment of mice with different pathogen associated molecular patterns

(PAMPs) as innate immune signals triggered full-blown anaphylaxis with a fast decline in body temperature and a significantly increased maximal temperature drop of around -4°C. In addition, PAMP pretreatment significantly reduced systolic blood pressure (-25, 38, 8 mmHg vs +1, 86, 1 mmHg following PBS pretreatment) and triggered high serum histamine levels (1740, 2 ng/ml vs 98, 727, 2 ng/ml following PBS pretreatment). Interestingly, not all PAMPs elicited co-factor dependent anaphylaxis as seen with the TLR2 ligands Pam2cys or Pam3cys, which had no effect. To identify the underlying mechanisms, extensive *in vitro* studies were carried out. However, mast cells generated from murine bone marrow or fetal skin as well as peritoneal mast cells showed no augmented mediator release in response to titrated doses of antigen in the presence of different PAMPs. Moreover, investigating mast cell deficient Kit w-sh/w-sh mice, we observed unaltered co-factor dependent anaphylaxis: In contrast to PBS pretreatment (Kit w-sh/w-sh: -1, 520, 39°C; WT:-1, 380, 44°C), PAMPs pretreated OVA sensitized Kit w-sh/w-sh mice (-4, 70, 87°C) showed the same highly significant temperature drop as did WT mice (-4, 060, 59°C).In addition, co-factor dependent anaphylaxis was completely abrogated following basophil depletion using the monoclonal antibody Ba103. In conclusion, we present for the first time a model, which allows investigation of co-factor dependent anaphylaxis. In this model innate immune signals were sufficient to elicit full blown anaphylaxis in response to low doses of antigen. Surprisingly, co-factor dependent anaphylaxis by PAMPs was independent of mast cells and fully dependent on basophils. Our results for the first time show a mechanism of how infections trigger anaphylaxis. This is of major clinical importance for the management and preventive measures in patients with co-factor induced anaphylaxis and may lead to new therapeutic strategies.

P124

Immunomodulatory effects on dendritic cells of biomaterial coatings based on artificial extracellular matrices (aECM)

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Induction of an adverse immune response towards an implant still represents the major threat of successful biomaterial application. As biomaterials can impact the host response the concept emerged to design 'immunomodulating' biomaterials. Various strategies have been developed to equip biomaterials with immunomodulating capacities. One strategy is the use of extracellular matrix (ECM) components as implant coating. Morphology and molecular composition of the ECM play a critical role in cellular migration and adhesion but also influence functions and responses of immune and tissue cells. Goal of biomaterial coating with ECM is to improve biocompatibility of implants and to accelerate wound healing by creating a naturally surrounding for the host cells.

In the present study we address the immunomodulatory effects of artificial ECM (aECM) that were generated utilizing the natural self-assembly potential of collagen in combination with either hyaluronic acid (HA) or chondroitin sulphate (CS). Both glycosaminoglycans (GAGs) were additionally modified by attaching of sulphate groups at low (S1) or high levels (S3) providing binding sites for endogenous growth factors and inflammatory mediators. Dendritic cells (DC) are key players of innate and adaptive immunity. They elicit immune responses but also possess immunoregulatory capacities. Activation of regulatory T cells by DC results intolerance induction and resolution of immune responses. In respect of biomaterial application action of such tolerogenic DC would provide a powerful mean of downregulating the inflammatory response at the implantation site. Since DC take an important part in biomaterial healing and integration we investigated the modulatory effect on DC maturation and function of different aECM.

Immature DC (iDC) were generated by culture of CD14+ monocytes for 4 days in the presence of GM-CSF and IL-4. The iDC were plated on a ECM or collagen matrices without GAGs that served as control. After 24 h the DC phenotype was assessed by analyzing expression of DC maturation surface markers, cytokine profile and allostimulatory ability in a mixed lymphocyte reaction. We find that collagen alone provokes DC maturation. Culture of iDC on collagen induces up-regulation of MHC molecules and co-stimulatory molecules (CD80, CD83, CD86) and release of TNF and IL-12p40, signals through which DC direct differentiation of T-cells towards an immune response. Of note, aECM attenuate the collagen driven DC maturation. Inflammatory cytokine release and expression of maturation marker was markedly down-regulated following DC interaction with a ECM. Moreover, DC maturation induced by LPS, a potent activator of DC, is also diminished in the presence of certain a ECM coatings as seen by reduced expression level of MHC and CD86 as well as decreased secretion of IL-12p40. Dentritic cells that have been prevented to mature are prone to develop a tolerogenic phenotype suggesting aECM to favor an immunosupressive DC response. T-cell responses are regulated by the extent of DC maturation as well as the DC phenotype. We therefore assessed T-cell priming in an allogeneic mixed lymphocyte reaction and found DC to differentially modulate T-cell proliferation depending on the underlying aECM substrate. The induced T-helper cell type responses are currently further characterized.

Our data clearly demonstrate the immunoregulatory capability of a ECM on DC and T-cell functions. Coating with a ECM therefore represents a powerful tool in the design of immunomodulatory biomaterials.

P125

Thymic stromal lymphopoietin enhances the Th22 inducing capacity of human plasmacytoid dendritic cells.

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Increased numbers of the recently defined IL-22 producing Th22 cells have been identified in inflammatory skin lesions, including atopic dermatitis. Thymic stromal lymphopoietin (TSLP) is classically present in atopic inflammation; however the impact of a TSLP-dominated inflammatory milieu on Th22 differentiation has not been analyzed till now.

Since human plasmacytoid dendritic cells (pDC) proved to be superior to other DC subtypes in priming Th22, the phenotype of activated human pDC and their priming effect on naive Th cells was analyzed as a function of TSLP.

Immature pDC and control DC were isolated from peripheral blood of healthy donors. Toll-like receptor (TLR) expression was then determined by quantitative real-time PCR and DC were activated with the respective TLR ligands. CPG2006 activation of pDC via TLR9 resulted in IL-6 and TNF-alpha production, as detected by intracytoplasmic FACS-analysis. However, the presence of TSLP strongly and dose dependently enhanced TNF-α and especially IL-6 production in pDC. To determine functional consequences of TLSP conditioned pDC, co-cultures of pDC and T cells were set up. TSLP treated pDC but not control DC orchestrated naive T cells to produce enhanced IL-22 and even reduced IFN-γ levels upon T cell restimulation. Th22 induction by pDC could be further upregulated by adding exogenous IL-6 and TNF-α. This effect was not proliferation-biased, as all co-culture conditions showed equal proliferation, as detected by H3-thymidin incorporation.

Our data provide further insight into the key role of IL-6 in the Th22-priming and clearly show that there is a regulatory impact of IL-6 in human pDC that is orchestrated by TSLP. This indirect Th22 polarizing capacity of TSLP is of relevance in regard to the role of TSLP in atopic diseases and may explain the presence of Th22 atopic skin lesions.

P126

Novel method of toxicity testing in zebrafish identifies detoxification of snake venoms by human skin mast cells

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Snakebite is responsible for substantial morbidity and mortality worldwide, primarily in poor, rural populations of Africa, Asia, Oceania, and Latin America. The World Health Organization lists it as one of 15 neglected tropical diseases that should be made a public health priority. Previously, we have shown that mast cells (MC) significantly reduce snake venom-induced pathology in mice, at least in part by protease-mediated degradation of venom components. To discover whether the same effect can be observed in primary human skin MC we have isolated MC from human skin and exposed them to the whole venoms of five distinct clinically relevant snake species. All of the venoms were found to induce MC degranulation, as measured by beta-hexosaminidase and histamine release. The percentage of MC degranulation was dose-dependent and was highest for *Daboia russelii* venom (max release 46 ± 7%) and lowest for the venom of *Echis carinatus* (max release23 ± 4%).To further assess whether the activation of MC leads to a detoxification of the venom, we next developed a novel application of toxicity testing using zebrafish (*Danio rerio*) larvae. With this method we were able to replace the extensive mouse experiments usually needed for assessing the toxicity of each venom. Using this assay, we show that degranulated human MC significantly reduce mortality in zebrafish larvae. Furthermore, the fatal effects of venom are completely neutralized by co-incubation with human tryptase. To assess whether the observed loss of toxicity is due to a degradation of venom components, we used the well-characterized venom from *Apis mellifera* (honey bee) and analyzed the venom by MALDI-TOF/TOF mass spectrometry. Using this method, we saw an effective degradation of the major bee venom components apamin, mcdp, and melittin by human MC and, to some extent, by human MC-derived tryptase. Our findings show that human skin MC are activated by and detoxify snake venoms and indicate that human recombinant MC proteases may be a promising non-species-specific antidote to the local cytotoxic, hemorrhagic, neurotoxic, and myotoxic effects of snake envenoming.

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Syndecan 1 and Syndecan 4 affect the severity of contact hypersensitivity in mice

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Dendritic Cells (DC) play an important role in the induction of an immune-response and the maintenance of immunological tolerance. The antigen-dependent activation and migration of DC in peripheral tissues is closely associated with interactions with the Extracellular Matrix (ECM). Syndecans (SDC) are transmembrane proteoglycans with heparin sulfate side chains. SDC act as integrin co-receptors and sequester extracellular signals like cytokines, thereby affecting cell migration.

We investigated SDC 1 and SDC 4-knockout mice compared to wildtype mice in the murine contact hypersensitivity (CHS) model.

Wildtype-mice, SDC4-/- and SDC1-/- mice were sensitized by 7% TNCB painted on the shaved abdomen. Five days later the mice were challenged with 1% TNCB and the induced ear swelling was measured by micrometer. The SDC1-/- mice showed an enhanced ear-swelling while SDC4-/- an early CHS response compared to wildtype. This can be partly explained by decreased DC migration in SDC 4-/- mice and enhanced migration in SDC 1-/- mice.

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Generation of a DEC205+ specific single chain fragment variable (ScFv) toxin to deplete tolerance inducing dendritic cells

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CD8+ DEC205+ CD11c+ Dendritic cells (DC) are able to cross present antigens via MHC-I and are involved in the induction of FoxP3+ Treg. In order to study their function in more detail, we set out to devise a novel tool to deplete this DC subset *in vivo*. We generated a single-chain fragment variable (scFv) specific for the murine DEC205 surface antigen (CD205). This scFv was fused to *Pseudomonas aeruginosa* Exotoxin A (ETA), an inhibitor of the cellular protein synthesis, leading to induction of apoptosis.

The respective DNA sequences were cloned into a bacterial 6xHis- and c-myc-tag containing vector and the bacterial expressed recombinant scFv fusion proteins were affinity-purified. As controls, a nontoxic DEC205-specific scFv and a β-Gal specific scFv fused to ETA were generated. All recombinant constructs were expressed in *E. coli* TG1 and protein purification was achieved by affinity chromatography. To assess targeting capabilities of the cloned constructs, cytospins of bone marrow derived (BM) DC were incubated with scFv and stained thereafter with myc-tag specific antibodies. Evaluation via fluorescence microscopy showed effective binding of the DEC205 specific scFv constructs. To reveal functionality of the anti-DEC205-ETA scFv, DC were incubated with graded doses of the scFv and induction of apoptosis was analyzed. Here a significant depletion of DEC205+DCwas achieved by treatment with anti-DEC205-ETA scFv but not with control scFv. In addition we could find that depletion efficacy was even higher with matured BMDC, where upregulation of DEC205 occurs. Specificity of the toxic effect was validated by incubation of DEC205- fibroblasts with anti-DEC205-ETA scFv that did not lead to apoptosis.

Thus, our data show that anti-DEC205-ETA scFv is an efficient tool for the depletion of DEC205+ DC and further experiments will reveal the role of this DC subpopulation in tolerance and immunity.

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Modification of monocyte-derived dendritic cells by physiologically relevant thermal stress implies high potential to improve current vaccination protocols

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Dendritic cells (DCs) are the most potent antigen-presenting cells to induce tumor-antigen specific immune responses and have therefore been frequently implemented in the development of anti-cancer vaccination protocols. However, while specific immune responses to tumor-associated antigens have been successfully induced in a considerable number of patients, these responses have not always been sufficient to reproducibly and consistently mediate useful anti-tumor clinical activity. Hence, methods to improve DC immunostimulatory function are continuously being optimized. Considering the favorable survival benefits achieved in cancer patients treated with hyperthermia, we developed a concept for the *in vitro* heat treatment of human monocyte-derived DCs (moDCs) resulting in an efficiently enhanced biological cell activity and function. For this purpose human moDC were exposed to a phys-

iological mild thermal temperature of 40°C for 24 h and then analyzed for (i) expression of different heat shock proteins, (ii) survival, (iii) expression of cell surface maturation markers, (iv) cytokine secretion, (v) migratory capacity as well as (vi) for their ability to prime nave CD8+ T cells after loading with MelanA peptide, transfection with MelanA-RNA or transduction with an adenovirus coding for MelanA. The results clearly indicate that in comparison to control moDCs, which remained at 37°C, heat treated DCs show no differences concerning their survival or their migratory capacity. Importantly, expression of the immune-chaperone heat shock protein 70 (hsp70) is clearly enhanced in heat-treated DCs. This effect is accompanied by an increased expression of co-stimulatory molecules such as CD80, CD83 and CD86 on the cell surface as well as by a markedly improved capacity to prime autologous naive CD8+ T cells *in vitro*, which is clearly the most important assay in respect to the physiological relevance of heat-activated DCs. Noteworthy, this effect is independent of the order of antigen-loading and heat treatment, as moDCs which were first loaded with MelanA antigen by adenoviral transduction and then heat shocked showed similar results when compared with moDCs which were first exposed to 40°C and then transduced with MelanA-specific RNA or loaded with MelanA peptides. Thus, heat shock represents an inexpensive and fast tool to boost the immune-stimulatory function of DCs, and could therefore be an interesting new strategy for cancer therapy, especially in combination with current immunotherapy protocols.

P130
Soluble CD83 promotes tolerance induction to skin and 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 that sCD83 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× with sCD83 (100 g/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 withanti-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 g/mouse, day-1–28) attenuated acute rejection and doubled heart graft survival to 15 1.0 days. In addition, sCD83 has synergy with sub therapeutic dose of eitheranti-CD45RB mAb (50 mg/mouse, day 0–13, i.p.) or Rapamycin (2 mg/kg, day 0–13, p.o.) to further improve graft survival to 32 3.6 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.

P131
Influence of specific OPN binding motifs on dendritic cell functions by nanostructured, biofunctionalized surfaces

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Background: Within the skin, cells sense structure, composition and stiffness of their environment by integrin-extracellular matrix (ECM) interactions, initiating bi-directional signaling that controls adhesion, migration, proliferation, differentiation and apoptosis. Osteopontin (OPN) contains a central RGD-peptide to interact with alpha V-integrins, known to mediate adhesion, migration and survival of cells. We and others found that RGD-integrin interactions of OPN aid to guide skin homing dendritic cells (DC) into lymph nodes and influences their Th-polarizing potential. Although OPN mediated signaling has been studied in a number of cell types, it is not know which cascades are induced in DC through OPN's specific peptide sequences and how physical ligand spacing on the nano scale affects signaling.

Methods and Results: RGD dependent integrin lateral clustering was studied using nanopatterned poly ethylene glycol (PEG) hydrogel surfaces, containing RGD-biofunctionalized gold nanoparticles on an otherwise inert background. By varying the inter-particle spacing, effects of integrin clustering on DC morphology, adhesion, migration, and function were studied. Adhesion was quantified with a computer-assisted model, migration monitored by time-lapse videomicroscopy, survival and activation by FACS, respectively.

DC adhere and survive on RGD-biofunctionalized nanopatterned surfaces for more than 48 h. FACS analysis comparing RGD biofunctionalized surfaces with the control nonsense-peptide RGE revealed anti-apoptotic effects of RGD-integrin ligation on DC. Furthermore, RGD-integrin ligation induced moderate upregulation of MHC II complexes and of the DC activation marker CD83.

Conclusion: We established a model to study the effects of specific OPN binding motifs on DC function under nano-range control of integrin ligation. This system allows for controlling integrin clustering through variation of the inter-particle spacing of RGD-binding motifs within a range of 20–200 nm and additionally enables to measure how alterations of cellular microenvironment stiffness can mimic different mechanical tissues properties. The established model will help to identify the code by which specific peptide sequences of ECM molecules like OPN influence and control immune cells. This may provide new approaches for preventive and therapeutic strategies to modulate inflammation in autoimmunity and allergic diseases.

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P132
Providing T-cell help to dendritic cells to induce better anti-tumor immune responses

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Up to now, cancer immunotherapy has mainly focused on the generation of tumor-specific CD8+ T cells, even though CD4+ T-cell help is required for a strong, 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. Here we investigated the antigen-specific T-cell/DC cross-talk by endowing CD4+ T cells with tumor-specific TCR. We observed an antigen-specific Th1 cytokine secretion, up-regulation of maturation markers (CD25, CD40, CD80, CD86, and CD70) on both immature and mature DC, and up-regulation of activation markers (CD25, CD69) on CD4+ T cells in a time-dependent manner after co-cultivation of TCR-transfected T cells with peptide-loaded DC. Transwell-assays revealed that, as expected, CD4+ T-cell activation was completely cell-cell-contact dependent, while the maturation of the DC was in part mediated by soluble factors. Pre-activation of CD4+ T cells led to an increase in IL12p70 secretion in co-cultures of T-cells and immature DC. Furthermore, we removed the CD4+ T cells by cell sorting after their antigen-specific (gp100 peptide) interaction with DC, and used the latter to stimulate nave CD8+ T cells via a MelanA epitope to mimic the sequential two-cell interaction model. To address the alternative three-cell interaction model all cell types were allowed to interact simultaneously. The three-cell interaction model resulted in superior CD8+ T cell expansion. Taken together, our findings provide new insights in the mechanisms of T-cell help and DC licensing.

P133
Antigen-specific stimulation of CD8+ T cells with dendritic cells causes reciprocal activation of both cell types

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The goal of immunotherapy of cancer is to initiate an effective adaptive immune response against the tumor. This involves the interaction of the antigen-presenting cell (i.e. the dendritic cell, DC) with both CD4+ and CD8+ T cells. Up to now, the exact mechanism by which these three cell types cross-talk is not yet fully understood, and it is especially unclear whether a bidirectional interaction takes place between CD8+ T cells and DC. Previously, we studied the cross-talk between CD4+T cells and cocktail-matured or immature monocyte-derived DC, and observed a clear up-regulation of maturation markers on DC and activation markers on antigen-specifically activated T cells accompanied by the secretion of Th1-type cytokines. In this study, we investigated whether CD8+ T cells are also able to talk to DC after their antigen-specific activation by antigen-loaded DC. For this purpose, we transferred a gp100/HLA-A2-specific T-cell receptor (TCR) by RNA-electroporation into CD8+ T cells, and co-cultured these cells with peptide-loaded DC. Antigen-specific stimulation caused an increased secretion of cytokines like IFNγ, TNF, and IL-2. Furthermore, we detected an antigen-specific up-regulation of activation markers like CD25 and CD70 on the CD8+ T cells and of maturation markers like CD25 and CD40 on the DC. Altogether the changes on the DC were, however, not as dramatic as after DC-CD4+ T cell-interaction. These data indicate that the antigen-specific cross-talk between DC and CD8+ T cell is also a bi-directional process, which leads to the maturation of the DC and to the activation of the T cells. However, CD8+ cannot fully replace the activation/licensing of dendritic cells by CD4+ T helper cells.

P134
Manipulating immune cell recruitment to the tumor by oncolytic viruses for combined immuno-virotherapy of malignant melanoma

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Melanoma is the most serious form of skin cancer. So far, overall success for treatment of advanced malignant melanoma is quite limited and new therapeutic approaches are needed. 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 however; adenoviral oncolysis 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 (oncolysate). To this end, we engineered an optimized melanoma-targeted oncolytic adenovirus (Ad5/3.2xTyr) that possesses strong tumor-selectivity and improved efficacy. So far it is not known if a specific anti-tumor immune response can be induced by oncolytic adenoviruses in humans. Here we focus on immune cell recruitment and activation by adenoviral oncolysis which essentially creates a vaccine against the cancer cells within the patient's body (oncolytic vaccination).

Previously, we showed that infection of cultured melanoma cells with Ad5/3.2xTyr did not induce significant changes in chemokine production. Correspondingly, endothelial cell-dependent and -independent recruitment of dendritic cells to melanoma cells was not affected by Ad5/3.2xTyr. However, we could show that recombinant human CCL5complements adenoviral oncolysate for recruitment of immune cells. In light of these results we are now investigating (i) if the tumor microenvironment has an impact on the production of immunomodulators during virotherapy of melanoma and (ii) if genetic modification of the oncolytic adenovirus can trigger the recruitment of immature dendritic cells (iDCs) to the tumor during viral oncolysis aiming at tumor vaccination.

To investigate the impact of the tumor microenvironment, we established living precision-cut tissue slices of melanoma biopsies. We showed that these living tissue slices are viable for >3 days in culture and could be infected by Adenovirus. Currently, we are analyzing the chemokine production before and after infection of these slices with Ad5/3.2xTyr and the results will be presented. To compensate the lack of change in immune cell recruitment during adeno-virotherapy we generated a genetically modified adenovirus encoding human CCL5/RANTES. Infection of melanoma cells with this virus resulted in secretion of CCL5 that possessed a bioactivity similar to bacterially produced recombinant human CCL5. Indeed, the recruitment of iDCs was increased after infection of melanoma cells with this CCL5-encoding adenovirus *in vitro*. We are presently analyzing the capacity of theseCCL5-recruited iDCs to phagocytose the oncolysate which would be a prerequisite for the induction of a systemic anti-tumor immune response. We hypothesize that the combination of tumor cell lysis and recruitment of DCs for oncolytic vaccination after infection of melanoma metastases with such genetically engineered oncolytic adenoviruses improves the clinical benefit of virotherapy.

P135
Reprogramming T cells with MHC-independent chimeric antigen receptors specific for MCSP

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T cells reprogrammed with a new specificity by introduction of a tumor-antigen-binding receptor are an innovative and promising tool to treat cancer. To overcome the MHC-restriction of wild type T-cell receptors (TCR), antibody-based chimeric antigen receptors (CAR) are an attractive alternative. We introduced such CAR transiently by mRNA electroporation to avoid persistent auto-aggression, which is a documented risk of engineered T cells with constitutive CAR expression.

CD4+ and CD8+ T cells were efficiently transfected with CAR consisting of different antigen-binding and signaling domains, specific for melanoma chondroitin sulfate proteoglycan (MCSP), which is, for example, expressed on most melanomas and some forms of childhood leukemias. The CAR-expression was transient and had disappeared at day 9 after electroporation. The reprogrammed T cells secreted cytokines upon specific stimulation with MCSP+ tumor cells and killed 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. Since the expression level can be regulated by varying mRNA quantities during electroporation, we now have the possibility to investigate how the affinity and the density of the receptor on the T cells affect antigen recognition, and how to avoid off-target specificity.
Taken together, this study shows a direct comparison of CAR with different scFv specific for the same antigen, and by using the RNA transfection technique and the choice of the CAR best suited for the immunotherapy of cancer is simplified.

P136
Designer DC as anti-melanoma vaccine: finding the right time to prime

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Electroporation of monocyte-derived dendritic cells (DC) with mRNA, to load them with antigen, or to manipulate their function, allows the generation of 'designer' DC for improved DC vaccination. In this study we examined various designer DC optimized in their T-cell stimulation capacity by introduction of different functional molecules. We directly compared DC electroporated at the immature stage with MelanA RNA alone, or a combination of MelanA RNA and non-optimized CD40L-,CD70-, and constitutively active TLR4 (caTLR4)-encoding RNAs (TriMix, as described by K. Thielemans et al.), to DC which were first matured (byIL-1β+IL-6+TNF+PGE2) and then electroporated with MelanA RNA alone, a combination of MelanA RNA and non-optimized CD40L RNA, or a combination of MelanA RNA and an optimized CD40L-encoding RNA (optCD40L). We investigated the kinetics of maturation marker expression after electroporation and found an up-regulation of CD40, CD80, CD86, and CCR7 in all conditions. Striking differences in OX40L, CD25, and CD83 expression were observed (especially at the 48 h time-point) between the DC transfected with Trimix compared to DC transfected with optCD40L. Furthermore, the production of the cytokines IL-12p70, IL-10, TNF, IL-6, IL-1β, and IL-8 after electroporation markedly differed between the differently matured DC, but had ceased for many of the cytokines after 24 h. Accordingly, we found that transfection of functional molecules enhanced MelanA-specific T cell expansion only transiently, and had largely disappeared after 24 h. Hence, we expect that DC, transfected after maturation and administered only 4 h later, will display superior immunogenicity in ongoing, two-armed DC vaccination trials.

P137 (V20)
Cutaneous pathogen associated molecular pattern (PAMP) induce systemic immune regulation mediated by myeloid-derived suppressor cells

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It is well established that Gram positive bacteria colonize the skin especially inflammatory skin lesions as in atopic dermatitis. It is not well understood how innate immune sensing of Gram positive bacteria influences cutaneous and systemic immune responses. In order to investigate consequences of microbial substances on the skin, contact hypersensitivity (CHS) to the hapten FITC was investigated. CHS to FITC is established by weekly applications of FITC to the shaved abdomen of mice and it is characterized by a Th2 dominated immune response and high IgE levels. Lipoproteins are important components of Gram positive bacteria and function asTLR2 ligands. Therefore we used synthetic TLR2 ligands Pam2Cys and Pam3Cys to mimic the presence of Gram positive bacteria on skin lesions. After the first FITC sensitizations, Pam2Cys and Pam3Cys were added during consecutive FITC applications. A consecutive FITC challenge at the ear skin leads to ear swelling with a peak at 24 h. This course of inflammation was not changed in mice that previously received Pam3Cys. In sharp contrast, in mice previously treated with Pam2Cys, ear swelling was significantly reduced by >80%. In addition, FITC-specific IgE and IgG1 antibodies and FITC-specific T-cell proliferation were also significantly decreased and these effects were TLR2 dependent. Investigating underlying mechanisms, we identified Gr1+CD11b+ myeloid derived suppressor cells (MDSCs) to be massively increased after Pam2Cys treatment only and this increase correlated with reduction of CD4+ and CD8+ T cells. Phenotypically, Gr1+CD11b+ cells wereCD16/32 (Fcγ-RIII/II)- and CD44-positive, and partially expressed MHC-II, F4/80, and B220. *Ex vivo* MDSCs effectively inhibited antigen specific T cell proliferation and cytokine production. This suppression was cell contact dependent and not due to T cell apoptosis. These regulatory functions of MDSC were partially mediated by inducible NO synthase (iNOS) and NO production as treatment of MDSC with iNOS inhibitor reduced the regulatory activity. Investigations with dendritic cells (DCs), previously cocultured with Pam2Cys-induced MDSCs, demonstrated an IL-10++semi-mature phenotype unable to potently stimulate T cells. Collectively, we show for the first time that the presence of certain lipoproteins on skin is sufficient to regulate systemic immune responses and that TLR2 ligands are capable to induce MDSCs. These data indicate that cutaneous Gram positive bacteria can have a profound impact on the hosts immune system. Further research is needed to characterize the natural source of the different lipoproteins, which may be pathogens of non-pathogenic commensals. These findings are crucial for the development of new therapeutic strategies for chronic inflammatory skin diseases.

P138
DEC-205 targeting of dendritic cells: a challenging approach of antigen loading

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Dendritic cells (DC) can be loaded with tumor antigen by targeting DEC-205 using antibody-antigen fusion constructs. We generated several constructs that consisted of a DEC-205-specific scFv antibody fragment linked to different T-cell epitopes from the tumor antigens MAGE-A1, MAGE-A3, and MelanA. These constructs specifically bound to monocyte-derived DC and were internalized independently of the DCs maturation status. We quantified the presentation of the MAGE-A3-derivedHLA-DP4-resticted epitope with specific T cells and showed that DEC-205-targetingwas significantly more effective than direct peptide-loading and electroporation of defined tumor-antigen RNA. We could also show the feasibility of our approach with DC from malignant melanoma patients. However, we detected a variety of possible sources of LPS-contamination during the production of the constructs, including sterile dialysis tubing and plastic ware. These LPS-contaminations can easily result in misleading data because DC, which naturally function as danger signal detectors, already reacted to traces of LPS. We detected that LPS-concentrations as low as 1 ng/ml induced changes in DC phenotype and led to secretion of IFN-gamma, which is often used as a readout when measuring antigen-presentation with T-cell clones. Other cytokines were produced at concentrations of 100 pg/ml or even less LPS. We also observed that the incubation of DC with LPS-contaminated constructs resulted in phenotypic changes and cytokine secretion in absence of specific T cells and in the negative controls, i.e. heat-denatured constructs and constructs containing a different scFv.
These findings confirm again the necessity to always perform properly designed negative controls.

P139
Generation of dual-specific CD8+ T cells against HIV-1 by transfection of TCR-encoding RNA

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HIV-1-specific CD8+ cytotoxic T lymphocytes (CTL) play an important role in the immune response against HIV. Unfortunately, many patients are unable to generate a strong immune response against this virus. For these patients, adoptive transfer of T cells reprogrammed with an HIV-specificity by TCR transfer is a potential immunotherapeutic strategy. However, the HI-virus can escape the immune response by mutating the epitope recognized by the TCR. In this study, we reprogrammed T cells by transfection of two different TCR specific for HIV-epitopes presented on different HLA-molecules simultaneously. The pressure of the dual-specific T cells on the virus should slow down the immune evasion. CD8+ T cells transfected with a Gag- and a Nef-specific TCR released cytokines (IL-2, TNF, and IFN-gamma) after antigen-specific stimulation. However, cytokine secretion and surface expression were inhibited by competitive effects between both TCR. To prevent mis-pairing and resulting competitive effects, the constant regions of the Nef-TCR were exchanged with murine constant regions. The murinization resulted in a higher expression and cytokine secretion when the Nef-TCR was introduced alone in the T cells, however, had no influence on the competitive effect in the dual-specific T cells. Cells reprogrammed with the murinized TCR, either introduced alone or in combination with the Gag-TCR, lysed antigen-loaded target cells more effective than T cells transfected with the human TCR. Interestingly, after antigen-specific stimulation of one of the TCRs on dual-specific T cells, this TCR was functionally down-regulated, without influencing the surface expression of the second introduced TCR. Furthermore, we were able to proof that both TCRs were efficiently transfected into the same CD8+ T cell. Taken together, CD8+ T cells, which were transfected with two different TCRs can represent a new tool to study TCR functionality and might be used in the adoptive immunotherapy of HIV-infected patients.

P140
Mast cells control cutaneous lymphocytic choriomeningitis virus infection in mice

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Mast cells (MCs) have been suggested to contribute to the immune response elicited by viral infections, most likely though their activation via PAMPs and TLR-mediated production of proinflammatory cytokines and chemokines, and have been shown to regulate CD8+ T-cell activities via TLR-3 stimulation *in vitro* and *in vivo*. Here, we demonstrate that MCs control cutaneous infection with the lymphocytic choriomeningitis virus (LCMV) by regulating CD8+ T-cell recruitment via LTB4 and promote CD8+ T-cell activity against viral epitopes. MC-deficient KitW-sh/KitW-sh mice intracutaneously infected with LCMV (5×104 pfu) exhibited decreased ear swelling responses as compared to normal Kit+/+ or MC-reconstituted KitW-sh/KitW-sh mice. Ear swelling after intracutaneous LCMV infection was accompanied by the recruitment of CD8+ T-cells. Treatment of mice with the5-lipooxigenase inhibitor BW A4C, which inhibits the synthesis of LTB4, significantly reduced the number of infiltrating CD8+ T-cells as assessed by FACS analyses and decreased ear swelling to the level of MC-deficient KitW-sh/KitW-sh mice. The analysis of systemic T-cell responses after LCMV skin infection revealed that the expansion of antigen-specific CD8+ T-cells assessed by tetramer staining was diminished in the absence of MCs. In addition, restimulation of lymph node-derived cells by the LCMV epitope gp33 resulted in dramatic reduction of IFNγ production byCD8+ T cells in KitW-sh/KitW-sh mice as compared to normal Kit+/+ orMC-reconstituted KitW-sh/KitW-sh mice. Thus, these data demonstrate that MC scrucially contribute to host anti-viral reactions and provide novel insights into the importance of MCs in modulating adaptive immune responses against viruses.

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Impaired T cell function in patients with chronic mucocutaneous candidiasis is independent from autoantibodies

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Chronic mucocutaneous candidiasis (CMC) constitutes a selective inability to clear infections with the yeast *Candida albicans* resulting in persistent debilitating inflammation of skin, nails, and mucous membranes. Recently it has been shown that CMC-associated IL-17/IL-22 deficiency is caused by autoantibodies to IL-17 and IL-22 in the serum. In order to characterise cellular immunity in patients with CMC including patients with autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED), we analysed the response of PBMCs to *Candida albicans* in an experimental setup with and without autoantibodies. Furthermore, differentiation to and effector-functions of Th17 cells – described to be involved in the clearance of Candida infections – were analysed. PBMCs of CMC patients ($n = 12$) and healthy controls ($n = 11$) were stimulated with Candida or PHA for 72 h with and without autologous serum. Moreover naive T cells from CMC patients and healthy controls ($n = 4$) were polarised to Th17 and Th1 cells; STAT3 activation in PBMCs after IL-6 stimulation was investigated in both groups ($n = 6$) by performing Trans AM. Cytokine production was quantified both in supernatants by ELISA or intracellularly by flow cytometry. Importantly, T cells from CMC patients secreted significantly lower amounts of Th17-associated cytokines IL-17A and IL-22 in response to Candida and PHA in both auto- and heterologous systems. Additionally, the incubation of nave T cells of CMC patients with a Th17-polarizing 'cytokine cocktail' revealed a reduced ability of Th17 differentiation and CMC patients exhibited a significantly decreased STAT3 DNA binding capacity compared to healthy controls. Our data indicate that detection of IL-17 deficiency in our heterologous system excludes a possible role of autoantibodies against IL-17 and Il-22 in our studies. The observed defect in STAT3 activation could contribute to an impaired Th17-polarization in CMC patients that could play a central role in the pathogenesis of chronic mucocutaneous candidiasis.

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Cytokine responses induced by *S. aureus* or *S. epidermidis* derived lipoteichoic acid differ significantly and are critically modulated byIL-4

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Staphylococci, especially *S. epidermidis*, are part of the endogenous human skin microflora not leading to inflammation under physiological circumstances. In contrast, colonization of *S. aureus* on atopic dermatitis (AD) skin leads to inflammation and disease exacerbation. Recognition of bacteria on the skin is mediated by the innate immune system. Activation of innate immune receptors has been shown to either promote or attenuate inflammation. During innate immune sensing cell wall components of Staphylococci are recognized by pathogen recognition receptors (PRR). The most important PRR ligands of Staphylococci are peptidoglycan, lipoproteins and lipoteichoic acid (LTA). To investigate innate immune sensing of pathogenic and non-pathogenic Staphylococci, highly purified LTA from *S. epidermidis* and *S. aureus* was prepared and characterized. Both LTA preparations induced maturation of dendritic cells (DC) as observed by upregulation of MHC II and B7-costimulatory molecules. DC activation was dependent on TLR2 and MyD88 asTLR2-deficient and MyD88-deficient DC remained immature. In contrast to DC maturation, marked differences were seen in regard to cytokine production. While *S. aureus* LTA induced large amounts of IL-12p70 in DC, IL-12p70 levels remained low in response to *S. epidermidis* LTA. Interestingly, production of the anti-inflammatory cytokine IL-10 was similar in response to both LTA preparations. To mimic atopic dermatitis skin, DC were activated in the presence of IL-4. IL-4 significantly enhancedIL-12p70 levels induced by *S. aureus* LTA in DC. In sharp contrast, low IL-12p70 levels induced by *S. epidermidis* LTA were further suppressed in the presence of IL-4. This immune modulation mediated by IL-4 was clearly dependent on STAT6 signaling as STAT6 knock-out DC failed to regulate LTA induced IL-12p70 levels in response to IL-4.

Our data demonstrate a hitherto undescribed functional difference between LTA from pathogenic *S. aureus* and from non-pathogenic *S. epidermidis*. It is of special interest that the innate immune sensing of LTA is mediated by TLR2 and MyD88 in both cases, however, these pathways lead to marked differences in regard to the induction of pro-inflammatory cytokines. Furthermore, the typical AD cytokine environment dominated by IL-4 modulates LTA mediated cytokine production with opposing effects, thus, providing an explanation how *S. aureus* leads to exacerbation of skin inflammation while *S. epidermidis* is well tolerated on the skin surface.

P143
Inhibition of reactive oxygen species (ROS) by methylene blue (Mb)-treatment protects mice from experimental arthritis

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Accumulating polymorphonuclear leukocytes (PMNs) at the site of inflammation characterize the pathology of autoimmune diseases such as rheumatoid arthritis. PMNs and consequently reactive oxygen species (ROS) play an important role in the pathogenesis of rheumatoid arthritis and thus can promote joint inflammation and joint destruction. Auto-antibodies against Glucose-6-phosphat-isomerase (GPI) induce arthritis in mice that closely resembles human psoriasis arthritis. The aim of our study was, to analyze whether inhibition of ROS by methylene blue (Mb)-treatment can minimize inflammation, angiogenesis and joint destruction in GPI-arthritis. The thiazine dye Mb has long been used to stimulate cellular redox metabolism by activation of pentose-phosphate-pathway. Therefore, Mb leads to development of NADPH which reduces glutathione and inhibits oxidative stress. In experiments, we injected GPI-serum in BALB/c mice to induce joint inflammation. We started daily intraperitoneal Mb-treatment (0.23 mg/kg body weight (BW)) or sham-treatment (PBS) 2 days after GPI-serum injection. To investigate the therapeutic effects of Mb we measured ankle thickness by using a micrometer. Moreover we analyzed glucose metabolism and hypoxia in arthritic joints, *in vivo* using the radio tracers 18F-Fluordesoxyglucose ([18F]FDG; glucose metabolism),18F-fluoroazomycin arabinosid ([18F]FMISO; inflammation-induced hypoxia) and small animal positron emission tomography (PET) as well as small animal computed tomography (CT) to gain anatomical information. Additionally we analyzed mRNA expression patterns of pro-angiogenic, and pro-inflammatory mediators as well as H&E stained slices of ankles of Mb-treated and sham-treated mice. Mb-treatment was started 2 days after induction of GPI-arthritis when the ankle thickness was still increased from 2.50.04 mm (day 0) to 2.80.15 mm (day 2). Therapeutic inhibition of arthritis was already detected 24 h after the first Mb injection, as the ankle swelling was reduced to 2.70.15 mm in Mb-treated mice compared to 3.20.16 mm in sham treated mice. Four days after onset of Mb-treatment (at day 6 after GPI-arthritis induction), Mb-treated mice displayed an unaltered ankle thickness of 2.70.13 mm while the ankle swelling in the sham-treated mice further increased to 3.60.06 mm. *In vivo* investigation of glucose metabolism by [18F]FDG-PET displayed a 51% reduction in 4 days Mb-treated mice compared to sham treated mice while hypoxia (detected by [18F]FMISO-PET) exhibited only a 23% reduction. H&E stained slices of arthritic ankles from Mb-treated mice confirmed strongly reduced angiogenesis, pannus formation, and joint destruction compared to sham-treated mice at day 7 after GPI-arthritis induction. Real-time PCR analysis of arthritic ankles 6 and 12 h after onset of Mb-treatment displayed an impressive reduced IL-6, IL-1β, IL-33, COX-2and MMP expression compared to arthritic ankles of sham-treated mice.

Thus, Mb-treatment can protect mice from joint inflammation, angiogenesis and joint destruction by inhibition of ROS. Thiazine dyes such as Mb might be of special interest for the development of new treatment strategies for psoriasis arthritis and other autoimmune diseases.

P144
Detection of IgE – Pemphigus autoantibodies against Desmoglein 3 by ELISA depending on serum dilution

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Introduction: IgE-autoantibodies (Abs) against Desmoglein 3 have been known for several years in patients with Pemphigus vulgaris (PV). Their relations in pathogenesis and clinical expression are likewise discussed.

Objectives: The detection of IgE-Abs in patients with bullous pemphigoids depends on serum dilution as described in literature. Analogous to patients with bullous pemphigoid we investigated the detection of IgE – Pemphigus autoantibodies (Dsg3) by ELISA in patients with PV for different serum dilutions (1:2, 1:10, 1:100 respectively). We were particularly interested in finding out if IgE-autoantibodies were present in patients without Pemphigus vulgaris.

Methods: Sera obtained from 15 PV patients between 2006 and 2008 as well as 20 sera of control patients (assumed but no confirmed PV) were measured for serum IgG and IgE levels of anti-Desmoglein 3 antibodies (ELISA MBL®, MBL Int., Biozol, Echingen, Germany) following serum dilution 1:2, 1:10, 1:100, respectively.

Results: In 14 of 15 patients with PV (93.3 %), IgG anti-Dsg3 antibodies (Abs) were detectable. Before performing ELISAs for detecting IgE anti-Dsg3 antibodies, we diluted the sera 1:2, 1:10, 1:100, respectively. In dilutions 1:2 and 1:100, significantly higher levels for IgE anti-Dsg3 Abs were found ($P < 0.05$, Mann–Whitney test) for patients with PV compared to the control group. The dilution 1:10 as well as undiluted sera showed no significantly higher levels for IgE anti-Dsg3 Abs for patients with PV compared to the control group. Therefore our further investigations focused on the serum dilutions 1:2 and 1:100. As there are no cut points for IgE anti-Dsg3 Abs levels, an optimum cut point was determined for each serum dilution based on the largest Youden index (= sensitivity + specifity-1) by ROC-curves. As it is not known if every patient with PV expresses IgE anti-Dsg3 Abs, attention was paid to a high value for specificity and negative predictive value for the test.

For serum dilution 1:2, an optimum cut point was set on 0.077 OD (optical density at 405 nm) and for serum dilution 1:100, an optimum cut point was set on 0.076 OD.

For serum dilution 1:2, 10 of 15 patients (66.7%) with PV expressed IgE anti-Dsg3Abs, whereas five of 20 patients (25%) of the control group showed positive OD values ($P < 0.05$, Chi-test). The specificity and negative predictive value were 75%, the positive predictive value and the sensitivity were 66.7%.

For serum dilution 1:100, 11 of 15 patients (73.3%) with PV expressed IgE anti-Dsg3Abs, whereas again five of 20 patients (25%) of the control group showed positive OD values ($P < 0.01$, Chi-test). The specificity was 75% and the negative predictive value was 78.9%. The positive predictive value was 68.8% and the sensitivity was 73.3%.

Conclusion: In our investigations we could demonstrate significantly higher levels of IgE anti-Dsg3 Abs by ELISA in serum dilutions 1:2 and 1:100 compared to the control group (assumed but no confirmed PV). Undiluted sera and the dilution 1:10 showed no significantly higher values for patients with PV. For dilutions 1:2 and 1:100 high values in sensitivity, specificity as well as positive and negative predictive values could be detected. Because of the optimum constellations of values, a dilution of 1:100 for our IgE anti-Dsg3 ELISA is recommended.

P145 (V24)
Route of T cell administration determines treatment efficacy in Th1 cell based Immunotherapy

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Introduction: Tumor-associated-antigen (TAA)-specific interferon-γ producing CD4+ T cells (Th1) can deploy strong antitumoral effects and play an important role in upcoming T cell based immunotherapy of cancer but require suitable administration routes to ensure appropriate homing to the target site. Treatment with intra-peritoneal (i.p.) administered TAA-specific Th1 cells arrest multistage carcinogenesis and prolonged life of pancreatic cancer bearing RIP1-Tag2 mice two-fold. Aims of our study were to determine whether i.p. administration of TAA-specific (Tag2)-Th1 cells is more effective than intra-venous (i.v.) administration and to analyze differences in Tag2-Th1 cell homing. Additionally we investigated differences in TAA-specific (OVA)-Th1 cell migration into exogenous intra-cutaneous B16 and OVA-B16 melanomas due to the route of OVA-Th1 cell administration.

Material and Methods: We injected i.p. or i.v. 1E+7 Tag2-Th1 cells or physiological saline (sham treatment) into RIP1-Tag2 mice starting at 6 weeks of age once weekly. Tag2-specific immunotherapy was monitored by *in vivo* measurement of the tumour size using a 7 T small animal magnetic resonance imaging (MRI) scanner and by detection of blood glucose levels (BGL). Additionally, we intra-cutaneously inoculated B16-melanoma cells into the left flank and OVA-B16-melanoma cells into the right flank of C57BL/6 mice 8 days prior i.v. or i.p. OVA-Th1 cell administration. CD4+T cells were isolated from Tag2 T cell-receptor transgenic (TCR-tg) mice orovalbumin (OVA)-TCR-tg (OT-2) mice and specifically cultured to generate a Th1phenotype. To investigate the route of T cell migration, Cy5 fluorescence labeled Tag2-Th1 cells were i.v. or i.p. injected in Th1 cell treated 17 week old RIP1-Tag2mice. The animals were sacrificed 5 days later. OVA-B16 and B16-melanomabearing mice were scarified four days after i.v. or i.p. administration of 1E+7Cy5-labelled OVA-Th1 cells. Tag2 or OVA specific Th1 cell migration and bio-distribution were investigated *in vivo* and *ex vivo* by optical imaging (OI) and FACS-analysis.

Results: At 14 weeks of age average tumor volume was 88 mm in sham treated RIP1-Tag2 mice, 7.4 mm in i.p. treated mice, while no tumors were detectable by MRI in i.v. treated mice. BGL in i.p. treated RIP1-Tag2 mice was 69 (18) mg/dl, at norm values in i.v. treated (129 (4) mg/dl), and <30 mg/dl in sham treated mice. H&E stained slices of pancreatic tissue from 17 weeks old RIP1-Tag2 mice showed large pancreatic tumors in i.p. treated but only small tumors in i.v. treated mice. The analysis of Cy5 labeled Tag2-Th1 cells revealed nearly no Cy5-Tag2-Th1 cell accumulation at the tumour site after i.v. administration but a strong accumulation 5 days after i.p. administration. I.v injected cells resided primarily in the lung and the spleen. Analysing OVA-Th1 cell migration in OVA-B16 and B16-melanoma bearing mice we detected a strong accumulation of the OVA-Th1 cells in the OVA-B16 and B16 tumours 4–5 days after i.p. administration, but not after i.v. administration. FACS analysis confirmed the optical imaging results.

Conclusion: Our data clearly indicate that i.v. administration of Tag2-Th1 cells is a much more efficient than i.p. administration and might completely block pancreatic tumor progression in RIP1-Tag2 mice. Most surprisingly, Tag2 and OVA-Th1 cells accumulate at the respective tumor site only after i.p. but not after i.v. administration.

P146
Myeloid 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 consistently detect TNF-α in sections of lesional psoriatic skin, but by the application of atyramide amplification system we obtained reproducible and firm stainings.

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.

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. Most TNF-α-producing monocytes expressed CCR2, whose ligands CCL2, CCL7 and CCL11 were strongly upregulated in lesional psoriatic skin.

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*, infliximab 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.

P147
Targeting of cytosolic pattern recognition receptors augments the efficiency of adoptive T-cell immunotherapy in a new mouse melanoma model.

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Activation of endosomal toll-like receptors by immunostimulatory nucleic acids is mandatory for regression of primary autochthonous melanoma following adoptive T-cell immunotherapy in our Hgf-Cdk4R24C mouse model. Here we targeted the cytosolic helicase MDA5 with dsRNA directly on melanoma cells to investigate whether it would augment adoptive T-cell therapy. To confirm direct effects

of dsRNA on melanoma cells in our model, we established the HCmel384 cell line from a primary Hgf-Cdk4R24C melanoma. HCmel384 grows *in vitro* with a dendritic, heavily pigmented phenotype. Upon transplantation in immunocompetent syngenic hosts, HCmel384 forms progressively growing, heavily pigmented melanomas, with little immune cell infiltration. These tumors morphologically resemble the primary autochthonous parental tumor.
Quantitative RT-PCR analyses show expression of the cytosolic nucleic acid receptors Rig-I and MDA5 as well as the endosomal TLRs 3 and 9 in HCmel384cells. The dsRNA mimic poly (I:C) complexed with polyethylenimine (PEI) targets cytosolic MDA5, leads to secretion of the interferon regulated chemokine CXCL10, upregulates MHC I and induces apoptosis of HCmel384. Targeting of endosomalTLR3 with naked poly (I:C) lacks these effects on tumor cells.
Based on these data, we reasoned that therapeutic targeting of MDA5 *in vivo* should promote direct melanoma cell death, support recruitment of cytotoxic T-cells in the tumor microenvironment and enhance melanoma cell recognition. Indeed, intratumoral injection of poly (I:C) complexed with PEI augments adoptive T-cell therapy in mice bearing established HCmel384 melanomas and enables complete regression of tumors in a significant proportion of mice. Because of the slow growth kinetics of HCmel384 we could assess the kinetics of the T-cell response and the generation and persistence of memory T-cells. Surprisingly, administration of poly (I:C) complexed with PEI does not affect the proliferation of adoptively transferred TCRtg T-cells, the acquisition of effector functions like interferon gamma production or the generation of memory T-cells, which is observed for the classical TLR ligands naked poly (I:C) and CpG.
Taken together, our data indicate that stimulation of cytosolic pattern recognition receptors augments the adoptive T-cell therapy of melanoma through direct effects on tumor cells *in vivo*.

P148
New lessons learned from cancer immunotherapy: tumor-specific CD4+ T cells frequently expand and react to short peptides

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The old paradigm says – short 9mer peptides bind to MHC class I and induce CD8+cytotoxic T cell (CTL) responses, whereas longer peptides are required to activate CD4+ helper T cells (TH) via MHC class II. Duly, most vaccination trials have been conducted with short 9mer peptides and the immunomonitoring was focused on the detection of the desired CD8+ CTL. Here we show that this is not at all the complete picture of anti-tumor T cell immunity.
Analyzing T cell responses against short 9mer peptides derived from different tumor antigens (Mage3, MelanA, Survivin) we did not only find the expected CD8+ CTL in our melanoma patients, but also quite frequently CD4+ TH cell responses. Interestingly, strong CD4+ TH responses were largely induced by MHC class I restricted vaccinations (DCs loaded with mRNA or peptide plus adjuvant). Testing the MHC-context in which the CD4+ TH cells do recognize short 9mer peptides revealed a clear MHC class II dependency. In many patients those class II restricted CD4+ TH responses were up to 10 fold stronger than expected CD8+ CTL responses and yielded cells capable of producing large amounts of IFNg, TNF alpha and IL2 mostly in a polyfunctional manner to stimulation with 9mer peptides.
In aggregate we provide evidence for a frequently overlooked polyfunctional class II restricted CD4+ TH response to small tumor peptides which role in cancer immunotherapy requires further attention.

P149
NF-κB-Inhibiting N-Acetylcysteine (NAC) protects from acute and chronic cutaneous delayed type hypersensitivity reactions (DTHR) by suppression of MMP-activity and angiogenesis

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Angiogenesis plays a major role in organ-specific autoimmune diseases caused by DTHR. NF-κB regulates the induction of pro-inflammatory mediators such as TNF in DTHR. Importantly, TNF enhances the expression of extracellular matrix degrading metalloproteinases (MMP) required for angiogenesis. Aim of our studies was to analyze whether inhibition of NF-κB is sufficient to suppress DTHR and whether analysis of MMP-activity is applicable to detect anti-angiogenic effects of NF-κB inhibiting agents *in vivo*. NF-κB-signaling is divided in a canonical and anon-canonical pathway. NAC suppresses both pathways by inhibition of IKKα, while BAY 11-7085 suppresses only the canonical pathway by interacting with IκBα.
In our experiments mice were sensitized at the abdomen and seven days later challenged at the right ear with trinitrochlorobenzene (TNCB) to induce and elicit acute cutaneous DTHR. Chronic DTHR was induced by repetitive TNCB-challenges every 2 days for up to five times. NAC- (5 mg/ml – drinking water) and BAY11-7085-treatment (50 μg– intra-peritoneally every 24 h) was initiated 2 days prior to the first ear challenge. We analyzed ear swelling responses 12–24 h after ear challenge and investigated MMP-activity *in vivo* using a MMP-2, 3, 9, and 13 activatable optical imaging (OI) probe. We injected the MMP-detecting OI-probe 12 h after the first, third and 5th ear challenge and performed *in vivo* OI-measurements24 h later. Additionally we examined H&E- and CD31-stained ear sections and mRNA expression patterns of pro-angiogenic, and pro-inflammatory mediators in ears tissue derived from NAC-, BAY 11-7085 or sham-treated mice.
Analyzing MMP activity *in vivo* by OI we detected a 4.0 fold increase compared to control ears after the first ear challenge. A further increase in signal intensity was observed during chronic DTHR. NF-κB-inhibiting NAC strongly reduced acute and chronic DTHR. Twenty-four hours after the first TNCB-challenge increase in ear thickness in NAC-treated mice was suppressed to 32% compared to sham-treated mice (6015 μm vs 19040 μm). Twelve hours after the third TNCB-challenge increase in ear thickness in NAC-treated mice was reduced to 51% (21515 μm vs 42020 μm) and to 42%(15540 μm vs 370150 μm) after the 5th TNCB-challenge. *In vivo* MMP activity in NAC-treated mice was reduced to 48% after the first, 78% after the third and 57%after 5th TNCB-ear challenge. H&E- and CD31-staining confirmed reduced inflammation and angiogenesis due to NAC-treatment. Real-time PCR analysis of ear tissue 4 h after the first, third, and 5th TNCB-challenge from NAC-treated mice indicated decreased MMP-2, 9, and 13 mRNA levels after the first and third but surprisingly increased mRNA levels after the 5th ear challenge when compared to sham-treated mice. As BAY 11-7085 suppresses only the canonical NF-κB pathway we could detect only faint therapeutic effects. Twelve hours after the third TNCB-challenge increase in ear thickness in BAY 11-7085-treated mice was reduced to only 79%(24680 μm vs 31065 μm 15 μm) compared to sham-treated mice.
Thus, NF-κB-Inhibition is a powerful therapeutic tool to minimize detrimental effects of acute and chronic cutaneous DTHR by suppression of MMP-activity and angiogenesis. Furthermore *in vivo* detection of MMP-activity by a MMP activatable OI-probe might be an applicable tool to monitor anti-angiogenic therapies of auto immune diseases such as rheumatoid arthritis.

P150 (V01)
Pathogenic T-bet+ Th17 cells develop in the absence of TGF-beta signaling

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Interleukin (IL-)17-producing CD4+ T cells (Th17) are critically involved in autoimmune diseases like psoriasis and multiple sclerosis. Crucial for Th17 cells *in vivo*, IL-23 has been thought to be incapable of driving initial differentiation. Instead, IL-6 and transforming growth factor (TGF)-β1 have been argued to be the factors responsible for initiating specification. Herein, we show that substantial Th17 differentiation can occur in the absence of TGF-β signaling. IL-23 receptor is rapidly expressed after initial stimulation with IL-6 and IL-23 and the combination of IL-6 and IL-23 with IL-1β effectively induced IL-17 production in nave precursors, independent of TGF-β1. Epigenetic modification of the Il17a/Il17f and Rorc loci proceeded withIL-23 in the absence of TGF-β1, allowing the generation of cells that co-expressed Rorγt and T-bet, which were more pathogenic in experimental allergic encephalomyelitis (EAE). In contrast, Th17 cells generated with IL-6, IL-1β and TGF-β1 expressed Ahr, IL-9 and IL-10. T-bet-positive Th17 only appear in the absence of TGF-β1-signaling and were also found *in vivo* during EAE. These data suggest a new model for Th17 differentiation and identify that pathogenic Th17 cells express ROR-γt, IL-23 receptor and T-bet.

P151
Peroxisome proliferator-activated receptor (PPAR) δ agonists induce endothelial ICAM-1 expression

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It is a known fact, that endothelial cells isolated from human tumors express much lower levels of adhesion molecules, that are involved in leukocyte vessel wall interactions, such as intercellular adhesion molecule-1 (ICAM-1). These mechanisms seem to be evolved by tumors to escape immunosurveillance. Therefore, the development of angiogenesis inhibitors that can make the tumor additionally more vulnerable for the immune system might be a new approach for the treatment of cancer. Peroxisome proliferator-activated receptor (PPAR) δ agonists display a variety of effects on pro- and anti-tumor processes. Recently, we could demonstrate, that PPARδ agonists induce pro-inflammatory cytokines in human endothelial cells and inhibit endothelial cell proliferation and angiogenesis. We now hypothesized that PPARδ agonists might also enhance the expression of ICAM-1 which would be an important prerequisite for tumor specific leukocytes to reach the tumor cells through the tumor vasculature.
We found that treatment with PPARδ agonists induced endothelial ICAM-1 protein expression in a time- and concentration-dependent manner. The expression of soluble ICAM-1 was not significantly affected by PPARδ agonist treatment. We also demonstrated that PPARδ agonists significantly induced accumulation of ICAM-1 mRNA. The treatment considerably induced transcriptional activity of 5′-deletionalICAM-1 promoter gene constructs. PPARδ agonist-mediated induction was conveyed by a GC-rich region, harboring one consensus Sp1 binding site. EMSA analysis demonstrated that constitutive Sp1-dependent DNA binding is increased by PPARδ activation.
Hence, the induction of ICAM-1 expression might represent a critical molecular mechanism which might be essential for a pro-immunogenic and therefore anti-tumorigenic effect of PPARδ agonists.

P152
Induction of anti tumor responses against malignant melanoma via antigen targeting *in vivo*

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Dendritic cells (DCs) are very important antigen presenting cells in the immune system. They are essential for the initiation of immune responses as well as for maintaining central and peripheral tolerance. By using chimeric antigen carrying antibodies directed against the DC-subset specific C-type lectin and endocytosis receptors DCIR2 (33D1) and DEC205, we are able to target antigens toCD11c+CD8- or CD11c+CD8+ DCs *in vivo*, respectively. We have demonstrated that the type of T cell response generated is dependent on the DC subset that presents the antigen *in vivo*. Here, we wanted to investigate if we can induce a protective anti-melanoma response by targeting DCs in nave animals *in vivo*. For inducing an efficient immune response antigen carrying antibodies 33D1 or DEC205 were applied under immunizing conditions. In the used murine melanoma mouse model and the used immunization protocol mice showed a mixed TH1/TH2 mediated antibody response and a strongly prolonged survival with a diminished tumor growth. Moreover, antigen targeting to both DC subsets induced an even better anti tumor response. Antigen targeting in a therapeutic setting induced a delayed tumor growth and prolonged survival. Our results show that antigen targeting of DCs might be a future option for the induction of protective anti-tumor responses.
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P153
Influence of titanium dental implants upon cytokine pattern of peripheral blood mononuclear cells (PBMC) exposed to titanium particles

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Potential hyperreactivity to dental titanium implants as elicitor of local complications (e.g. loosening/'sterile peri implantitis') is still a controversial issue. Thus, we wondered in a first set of experiments, if PBMC reactivity to titanium particles *in vitro* would differ between healthy individuals without and with (symptom free) titanium dental implants.
Ten healthy individuals without dental implants and five healthy individuals with well tolerated titanium dental implants took part in this experiments. PBMC were isolated from the peripheral blood by densitiy centrifugation and stimulated for 5 days *in vitro* with TiO2 particles (Rutile and Anastase structure) in different concentrations. The production of IL-1β, IL-10, IL-17A, TNFα and IFNγ in the cell culture supernatant was analyzed by multiplex cytometric bead assay.
As compared to baseline (cells with medium alone) PBMC of blood donors without titanium dental implants produced high levels of IL-1β on exposure to Rutile titanium particles (mean: 2355 ± 1052 fold) in comparison to the five blood donors with dental implants (mean: 4.7 ± 2.7 fold). Also TNFα and IFNγ production was more pronounced in the non-dental-implant group. IL-17A production was similarly low in both groups. Stimulation with tetanus toxoid and phorbole 12-myristate 13-acetate as positive controls led to similar cytokine production in both groups. Interestingly, only PBMC of dental implant bearing blood donors showed a baseline IL-10 production in the culture with medium alone.
To conclude from our ongoing experiments, an initial production of inflammatory cytokines seems to be a 'normal' response upon contact of PBMC to titanium particles. After long term *in vivo* exposure, e.g. after implantation of dental titanium based implants, the here observed reduced *in vitro* inflammatory mediator production may reflect (IL-10 mediated?) tolerance phenomena.

P154 (V14)

The role of regulatory T cells in an HLA-class II transgenic mouse model of pemphigus vulgaris.

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Pemphigus vulgaris (PV), a severe blistering autoimmune disease affecting the skin and mucous membranes, is strongly associated with the HLA-DRB1*0402 allel that is in linkage disequilibrium with HLA-DQA1*0301, HLA-DQB1*0302 (DQ8). The autoantibody (autoab) response to the major autoantigen in PV, desmoglein 3 (dsg3),is thought to be driven by autoreactive, dsg3-specific CD4+ T cells. Our group and others have previously identified and functionally characterized dsg3-reactive CD4+T cell clones from peripheral blood of PV patients. These T cell clones recognize immunodominant epitopes of the dsg3 protein in the context of the disease associated HLA-DRβ1*0402-molecule. Previous investigations suggest that type I regulatory, interleukin 10-secreting T cells in PV patients are significantly decreased compared with HLA-matched healthy individuals. HLA-DR0402-DQ8-human CD4-transgenic mice that are deficient for murine MHC class II (I-Aß-/-) provide an *in vivo* model for studying the CD4+ T cell and antibody (ab) response to human dsg3 protein. Previously, we have shown that immunizing HLA-DR0402-DQ8-transgenic mice with recombinant human dsg3 protein induces IgG ab that are pathogenic by inducing acantholysis in cultured human keratinocytes and human skin biopsies. Moreover, CD4+ T cells isolated form lymphoid tissues of dsg3-immunized HLA-transgenic mice, recognize HLA-DRβ1*0402-restrictedimmunodominant dsg3-epitopes, previously characterized in PV patients. In this study, we investigated the potential role of naturally occurring regulatory CD4+,CD25+, Foxp3+ T cells (Treg) in HLA-DR0402-DQ8-transgenic mice. To address this question, we applied the depleting anti-CD25 ab (PC61) and the anti-CD28 super agonist (anti-CD28SA) D665. While the anti-CD25 ab induced a dramatic decrease in CD4+, CD25+, Foxp3+ T cells in lymphnodes, spleen and peripheral blood, treatment of the HLA-transgenic mice with the antiCD28SA induced are markable expansion of Treg in these organs as determined by flow cytometry analysis. Furthermore, we investigated the effect of either Treg-depletion by PC61 a band Treg-expansion by anti-CD28SA, respectively, prior to immunization with human dsg3 protein. The cellular immune response to dsg3 was measured by proliferation assays using lymphocytes from draining lymph nodes of dgs3-immunized HLA-transgenic mice. Anti-CD25 ab treatment lead to an enhanced proliferative response to dsg3 whereas anti-CD28SA-treated animals showed a decreased dsg3-specific response *in vitro*. In accordance with these changes in the cellular compartment, we noticed enhanced titers of dsg3-reactive IgG in anti-CD25 ab treated mice. The humoral immune response to dsg3 was decreased in CD28SA pretreated HLA-transgenic animals. In summary, our results suggest an important role of Treg on both the cellular and humoral immune response to dsg3 in an HLA-transgenic mouse model of PV and these findings should prompt further investigations of the role of Treg in PV patients. Finally, restoring impaired mechanisms of peripheral tolerance to dsg3 in PV might represent an innovative therapeutical approach in this autoimmune disease.

P155

Therapeutic response to TNF-α blockade in psoriasis is associated with increased numbers of circulating regulatory T-cells, decreased Th1 and Th17cells in psoriatic skin and altered proliferative response of CD4+CD25+CD127low regulatory T-cells

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Psoriasis vulgaris is characterized by keratinocyte hyperproliferation and altered differentiation, augmented vascular permeability and an inflammatory infiltrate. Its etiology is still unknown, but it is generally believed to be a polygenic T-cell dependent chronic relapsing inflammatory autoimmune disease. The primary immunologic driving force for psoriasis is thought to be mainly Th1 and Th17 cells. Dysfunction of the CD4+CD25+CD127lowFoxp3+ regulatory T cell (Treg) population, which is crucial for the prevention of spontaneous autoimmune disease, however might be a potential explanation for unrestrained pathogenic effector T-cell proliferation in psoriasis. Animal models demonstrated that prepsoriatic skin implanted on to nude mice requires concomitant T-cell transfer to develop a psoriatic phenotype. Furthermore, treatments aimed at blocking immune responses have beneficial effects on the course of psoriasis. Recruitement of Th1 and Th17 cells in psoriatic skin leads to accumulation of TNFα which is critical for the initiation and maintenance of psoriasis.

This study investigated the dynamics of Treg populations, in peripheral blood and lesional skin of patients with severe plaque-type psoriasis ($n = 30$) through the course of treatment with immune-targeted anti TNFα therapies with adalimumab ($n = 15$) and etanercept ($n = 15$) over a time period of 28 weeks. We performed cytokine flow cytometry every 4 weeks using a FACSC alibur cytometer. For surface staining freshly lysed cells were stained with the following Ab-Pool: anti-human CD4-FITC, anti-human CD25-APC and PE-conjugated mouse anti-human CD127. Immunohistochemical staining procedures were performed at week 0 and 8 for CD4, CD8, CD45RO, Foxp3, CLA and IL-17. Furthermore CD4+CD25+CD127low Treg cells were isolated for functional characterization at week 0, 12 and 28. CD4+CD25-Teff cells were isolated by negative selection and co-cultured at a concentration of1 × 105 cells/ml with CD4+CD25+CD127low Treg cells at ratios of 1:1, 2:1, 4:1 and 8:1, respectively, in the presence of 4 × 105/ml autologous x-irradiated (2x31Gy) PBMC and anti-CD3. T cell proliferation in the co-culture experiments was assessed 5 days later by the uptake of [3H]-thymidine which was added for the final 18 h of culture.

Regarding the peripheral blood compartment in psoriasis patients treated with anti TNFα therapies, the CD4+CD25+CD127low T cells were elevated during 28 weeks in both groups. This was accompanied with a decrease of all examined T-cell subsets, including IL-17 producing cells and Foxp3+ T cell in epidermis and dermis of psoriatic skin lesions after 8 weeks, an altered proliferative response of CD4+CD25+CD127low regulatory T-cells after 28 weeks and a clinical response, documented with a significant decrease in PASI and DLQI during 28 weeks. We could confirm the crucial role of IL-17 in psoriasis as a Th17-cell-dependet chronic inflammatory dermatosis. Furthermore Treg degradation may contribute to psoriasis pathogenesis. Thus, TNF-α blockade has profound effects on Treg subsets in peripheral blood and psoriatic skin. Compensating for the low Treg count to a more favorable Treg balance, as we have demonstrated, could provide benefit for psoriasis patients.

P156

Systemic therapy of plaque-type psoriasis ameliorates endothelial cell function: results of a prospective pilot trial

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Background: Severe psoriasis is associated with significant cardiovascular mortality.

Aim: We investigated the effects of systemic therapy on the cardiovascular risk of psoriasis patients.

Methods: Thirteen consecutive patients receiving fumaric acid esters were included and followed for 24 weeks both clinically and by means of laboratory monitoring. Venous occlusion plethysmography of the forearm for measurement of the forearm blood flow (FBF) was performed, assessing both endothelium-dependent vasodilation through stimulation with acetylcholine, and endothelium-independent vasodilation through stimulation with sodium nitroprusside (SNP).

Results: Ten patients completed the study. Eight of these showed a PASI-50 response. Two of three patients with clinical insulin resistance (Homeostasis Model Assessment of insulin resistance >2.5) showed normal insulin responsiveness at the end of the study. Clinical improvement was paralleled by a reduction of high-sensitive CRP serum levels (median: −25%). There was a trend towards reduced serum levels for the vascular endothelial growth factor (median: −10%) and resist in (median: −4%), while the potentially cardio-protective adiponectin showed a trend towards increased serum levels under therapy (median: +19%). To assess systemic vasodilator function, patients underwent plethysmography prior to initiation as well as after 24 weeks of continuous therapy. Systemic therapy with fumaric acid esters was associated with a significant increase in endothelium-dependent ACH-mediated FBF after 24 weeks. In contrast, endothelium-independent SNP-mediated vasodilation was not affected.

Conclusion: This is the first prospective study documenting an amelioration of endothelial cell function in patients with moderate-to-severe plaque-type psoriasis under effective continuous systemic therapy. Future studies need to compare the cardio protective effects of different treatment modalities, based on hard end points such as the rate of myocardial infarction.

P157

Microdialysis documents changes in the micromilieu of psoriatic plaques under continuous systemic therapy

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Background: Microdialysis is a novel technique suitable to analyze soluble mediators in the skin compartment.

Aim: We applied this methodical approach to monitor changes in the micromilieu of psoriatic plaques under therapy.

Methods: Tissue fluid was collected from lesional and non-lesional skin of three patients with severe plaque-type psoriasis prior to as well as after 12 weeks of continuous oral therapy with fumaric acid esters. Concentrations of a spectrum of cytokines and adipokines were measured using a commercial fluorescent bead immunoassay and compared to the respective values in the patients serum samples.

Results: All patients tolerated the treatment well, there were no adverse events necessitating a modification of the treatment plan. Two patients responded well to the treatment, achieving a PASI-50 and PASI-75 response, respectively, while the third patient showed a PASI reduction of only 3.4 points, corresponding to a 35% improvement of his initial PASI. When analyzing the tissue fluids collected through microdialysis prior to initiation of therapy, we found higher levels of all mediators studied in lesional when compared to non-lesional skin, the only exception being adiponectin. Among the cytokines analyzed, the highest concentrations were observed for interleukin 6 (IL-6) (mean: 184 ng/ml), followed by IL-23 (mean: 151 ng/ml) and IL-18 (mean: 131 ng/ml), while IL-2 concentrations were found to be about one log lower (mean: 17.7 ng/ml). The concentrations of the respective mediators in non-lesional skin at the same time were found to be statistically significantly lower. A similar distribution pattern was observed for the adipokineresistin, while adiponectin showed higher concentrations in tissue fluid from non-lesional skin. Tissue fluid collected from lesional psoriatic skin after 12 weeks of continuous treatment with fumaric acid esters exhibited significantly lower levels of the cytokines IL-6, IL-18, and IL-23, while this effect did not reach statistical significance for IL-2. Again, the corresponding concentrations in non-lesional skin were found to be lower. This difference, however, was not statistically different. Resistin measurements yielded similar results as the cytokine analyses. For adiponectin, the changes were heterogeneous: Its concentration in lesional skin increased in one patient, while it deceased in the other two. The respective values equaled those of non-lesional skin at that time.

Conclusion: We were able to demonstrate through microdialysis a shift in the micromilieu of psoriatic plaques, characterized by reduced levels of pro-inflammatory mediators in patients under effective systemic anti-inflammatory therapy. This approach is suitable to more directly study the pathomechanism causing the psoriatic phenotype in general and insulin resistance in the skin compartment in particular.

P158

Successful treatment of scleroedema adultorum Buschke (SAB, a rare post-streptococcal complication) with intravenous immunoglobulin (IVIG)

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A 38-year old female patient presented with a 2-week history of a rapidly progressing symmetric induration of the skin over her neck, shoulders, chest and face. Thoracic excursion, shoulder joint motility and movement of the head were substantially impaired. Additionally, a papular rash was observed on the extensor surface of upper arms. The patient reported a severe upper respiratory tract infection requiring antibiotic treatment that had occurred approximately 7 weeks before the onset other skin symptoms.

A detailed history and laboratory analysis did not reveal any evidence of hematologic malignancies such as multiple myeloma, diabetes mellitus, autoimmune diseases or any ongoing infection with the exception of an elevated anti-streptolysin O antibody titer of 658 IU/ml (normal range <200 IU/ml). A complete blood count including differential was in the normal range. Histopathology from two biopsies taken from the right chest and upper arm demonstrated marked sclerosis of the dermis, absence of mucin deposits and a lymphocytic infiltrate without eosinophils.

Based on the history, the clinical presentation and the histopathological findings a diagnosis of SAB was established. Treatment was initiated with oral methylprednisolone (initial dose of 1.5 mg/kg) in conjunction with medium-doseUVA-1 phototherapy and physical therapy over a period of 4 months. This resulted in an arrest of disease progression but no improvement of the patient's condition. Subsequently a therapy with IVIG (2 gr/kg body weight every 4 weeks for five cycles) was started. There was a rapid improvement of the patient's condition starting after the first IVIG administration.

SAB is a rare disorder manifesting as non-pitting induration of the skin that starts on the head and spreads to other areas of the body. Extracutaneous organs such as the muscles, joints, heart or eyes may also be affected. The exact pathomechanism is incompletely understood. SAB is frequently associated with streptococcal infections, hematologic malignancies such as multiple myeloma or diabetes mellitus. Therapeutic options include irradiation with PUVA, UVA-1 or electron-beam, cyclosporine, cyclophosphamide and oral corticosteroids but often are of limited benefit. Here we propose IVIG as an alternative therapy option for SAB patients.

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Differential expression of antimicrobial peptides in synovial tissue of psoriatic arthritis indicating a diverse pathogenetic concept of skin and joint disease?

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Antimicrobial peptides (AMP) are highly active proteins with a broad-spectrum microbiocidal activity. Several members of this protein family are considered to have also immunomodulating functions. Therefore, certain AMP may play a role in the pathogenesis of inflammatory skin and joint diseases such as psoriasis and psoriatic arthritis where the pathogenesis is not yet understood. The aim of this study was to investigate the expression of various classes of AMP in human synovial tissue derived from patients with psoriatic arthritis in comparison to other forms of arthritis and healthy controls.

Synovial tissue samples derived from involved knees of patients with psoriatic arthritis (PsA, $n = 9$), rheumatoid arthritis (RA, $n = 13$) and osteoarthritis (OA, $n = 5$) as well as from healthy controls ($n = 5$) were included into this study. Immuno histochemical staining was performed with specific antibodies for psoriasin (S100 A7), RNase 7, human beta-defensin (hBD)-2 and -3, cathelicidin/LL-37, S100A8, S100 A9, and human neutrophil peptides (HNP)1-3.

Highest immunoreactivity for S100 A8 and A9 was found in tissue samples derived from patients with PsA and RA with a more pronounced pattern of S100 A9 in PsA. Synovia from OA and healthy controls only demonstrated low activity for these AMP. For HNP1-3 intensive staining was found in OA, followed by healthy controls, RA and PsA, with positive cells in the synovial layer especially in PsA and RA tissue. Moderate immunoreactivity could be demonstrated for LL-37 in PsA, OA and rarely in RA, whereas all control samples were negative. Expression of hBD-3 was restricted to vessels within individual specimen. Interestingly, no synovial tissue immunoreactivity was demonstrated for psoriasin, RNase 7, and hBD-2.

In conclusion, S100 A8, S100 A9, HNP 1-3, and LL-37 are highly upregulated indifferent forms of arthritis with a distinct pattern for PsA. In contrast to psoriasis vulgaris, a skin disease with a well known intensive upregulation of several AMP classes, no expression of psoriasin, RNase 7, hBD-2, and -3 could be demonstrated in the synovial tissue of patients with arthritis.

The new findings may help to further elucidate the role of antimicrobial peptides in the pathogenesis of psoriatic skin and joint disease.

P160
A novel ELISA for the sensitive and specific detection of IgA auto antibodies against the ectodomain of collagen XVII/BP180

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Collagen XVII/BP180 is a major target of autoantibodies belonging to the IgG, IgE and IgA isotypes in pemphigoid diseases. In the lamina lucida-type of linear IgA disease, the auto reactivity against the epidermal basement membrane is associated with IgA autoantibodies against collagen XVII, which preferentially react with its shed ectodomain. While for the detection of IgG and IgE autoantibodies specific to collagen XVII several ELISA systems have been established, no quantitative immunoassay has been yet developed for IgA autoantibodies. Therefore, the aim of the present study was to develop an ELISA to detect IgA autoantibodies against collagen XVII in the sera of patients with pemphigoids. For this purpose, we expressed a soluble recombinant form of the collagen XVII ectodomain in mammalian cells. IgA autoantibodies from patients with linear IgA disease recognized both the recombinant and the soluble keratinocyte-derived ectodomain of collagen XVII by immunoblot analysis. ELISA test conditions were determined by chessboard titration experiments. Subsequently, the optimized assay was carried out using sera from patients with linear IgA disease ($n = 27$) and healthy donors ($n = 30$). By receiver operating characteristics (ROC) analysis, an area under curve (AUC) of 0.9123 was calculated, indicating an excellent discriminatory capacity. Thus, a sensitivity and specificity of 82% and 87%, respectively, were determined for a cut-off point of 0.065. As additional control groups, sera from patients with bullous pemphigoid ($n = 32$) and dermatitis herpetiformis ($n = 50$), a disease associated with IgA autoantibodies against transglutaminase, were tested. Surprisingly, in 60% of bullous pemphigoid patients IgA autoantibodies recognized the ectodomain of collagen XVII. Four of 50 (8%) of dermatitis herpetiformis patients sera slightly topped the cut-off value. In conclusion, we developed for the first time an ELISA for the specific and sensitive detection of serum IgA autoantibodies specific to collagen XVII in patients with pemphigoid diseases. This immunoassay should prove a useful tool for clinical and translational research and should essentially improve the routine diagnosis and disease monitoring in linear IgA disease. Moreover, our findings strongly suggest that linear IgA disease and bullous pemphigoid represent two ends of the clinical spectrum of an immunological loss of tolerance against defined hemidesmosomal proteins mediated by both IgG and IgA autoantibodies.

P161
Vaccination with class I and II tumor peptide-loaded, cocktail-matured monocyte-derived DCs demonstrates solid immunogenicity and prolonged survival in a subset of melanoma patients exhibiting a particular gene expression profile

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We have intentionally used so far 'GM-CSF + IL-4' monocyte-derived dendritic cells (DCs), matured by the cocktail consisting of IL-1beta + IL-6 + TNF alpha + PGE2, in all of our trials using peptide-loaded DCs to get solid insight into their immunogenic properties by extensive serial immunomonitoring at each vaccination time point. In a 62 patient trial which has now matured (last patient out June 2007) in metastatic melanoma, tumor-peptide specific IFN gamma producing CD4+ T cells were regularly detectable *ex vivo* by Elispot. This is surprising given the low IL-12p70 release from cocktail-matured DCs *in vitro* but may be explained by their expression of CD70. Vaccine-specific CTL were, however, only weakly detectable *ex vivo*, but the CTLp frequency was markedly increased when measured by standardized, tetramer-based MLPC assays, and a substantial proportion of these CTL were of high affinity, polyfunctional and lysed even autologous tumor cells.

We also observed a markedly prolonged survival which in stage IV melanoma patients (defined >24 months) appeared to require both a 'strong' induction of immunity in the first 3 months and a 'friendly' transcriptome pattern (e.g. up regulation of T cell markers, chemokines, innate immune factors) in pre-vaccination metastases.

Even though objective regressions were rare median overall survival in stage IV patients has continuously increased in our consecutive trials from 8 and 11 months (mono-peptide DC trials) to 24 (multi-peptide, low-dose DC, i.e. multiple class I and II peptides, 4 million DC per class I peptide) to 46+ months in the current higher-dose (10 million DC per class I peptide used) multi-peptide DC trial.

P162
Susceptibility of pathogenic and commensal Staphylococci to skin-derived antimicrobial proteins

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Human skin releases several antimicrobial proteins (AMP) which contribute to protect the skin against infection. *Staphylococcus aureus* (*S. aureus*) represents an important pathogenic gram-positive bacterium associated with several skin infections. Recent work has shown that AMP especially human beta-defensin (hBD)-3 and RNase 7 help to control skin colonization with *S. aureus*. Other Staphylococci such as *S. epidermidis* are part of the commensal skin flora. However, the role of AMP in regulating the commensal flora is still emerging. Aim of this study was to gain more insight into the capacity of AMP to control the growth of commensal skin bacteria. Therefore we performed a systematic comparison of the susceptibility of *Staphylococcus aureus* and various commensal Staphylococci to the important skin-derived AMP human beta-defensins (hBD)-2 and -3, RNase 7 and psoriasin (S100A7). We verified that both, hBD-3 and RNase 7 exhibit potent activity against *S. aureus*. Both AMP were also highly effective in killing commensal strains of *S. lugdunensis*, *S. warneri*, *S. hominis*, *S. cohnii* and *S. haemolyticus*. HBD-2 exhibited also a potent activity against these commensal Staphylococci strains with reduced activity compared to hBD-3 and RNase 7. HBD-2 was also active against *S. aureus*, but higher concentrations were required for efficient killing. Whereas hBD-3 and to a lesser degree also hBD-2 effectively killed the abundant commensal *S. epidermidis*, RNase 7 exhibited very low activity against *S. epidermidis*. Psoriasin, the most abundant AMP on the skin surface, barely killed *S. epidermidis*. Other commensal Staphylococci as well as *S. aureus* were also affected only at high concentrations of psoriasin.

In summary, our data revealed that hBD-2, hBD-3 and RNase 7 are active against *S. aureus* as well as against various commensal Staphylococci suggesting that these AMP may limit the growth of pathogenic bacteria such as *S. aureus* besides controlling the commensal Staphylococci flora. It remains to be determined whether the weak activity of RNase 7 against *S. epidermidis* may explain the abundance of *S. epidermidis* on human skin. The low activity of psoriasin against Staphylococci is inline with recent data describing psoriasin as an AMP with preferential antibacterial activity against *E. coli*. However, since psoriasin is the most abundant AMP on skin surface and may act in synergy with other AMP further studies have to verify the hypothesis that psoriasin has no major function in controlling the growth of Staphylococci.

P163
Multi epitope ligand cartography reveals new insights in the role of Notch signalling in skin diseases

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In the skin Notch signaling may regulate homeostasis by balancing cellular processes of proliferation, differentiation and survival or apoptosis. Here we demonstrate the analysis of Notch signaling in Psoriasis, a chronic skin disease that is characterized by hyperproliferation and aberrant differentiation of keratinocytes by means of Multi Epitope Ligand Cartography (MELC) Technology. This high-dimensional fluorescence microscopy is based upon a repeated cycling of a skin tissue section through (i) incubation with a flurophore-labeled antibody, (ii) fluorescence imaging and (iii) soft bleaching. This method allows to stain one and the same tissue section with up to 100 fluorescent markers and to analyze their combinatorial expression. We compared skin biopsies from psoriasis with healthy normal skin. A library of 30 fluorescent markers was applied to visualize some of the main players of the Notch pathway. Our results now indicate that Notch signaling is profoundly disturbed in psoriatic lesions compared to healthy skin. Using the MELC Technology we were also able to further characterize up- and downstream effectors of Notch receptors by staining, for example, for metalloproteases, p63, Wnt and Sonic hedgehog (Shh). Our analysis shows that the MELC Technology is a novel and invaluable tool to analyze cell signaling in tissue samples, which had not been possible previously.

P164
Characterization and quantification of inflammatory markers in lesional and non-lesional skin of psoriasis via microdialysis

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Objectives: Kutaneous Microdialysis is an *in vivo* method for detection of soluble mediators in the interstitium. Psoriasis is a recurrent inflammatory disorder of the skin. The aim of this study was using cutaneous microdialysis to analyze different mediators in the interstitium of the dermis in lesional and non-lesional skin of 14 volunteers.

Methods: Fourteen patients with moderate plaque psoriasis (PASI 12.36.1; DLQI 17.65.5) at upper or lower extremities were included in a clinical trial (PASI 12.71.7; DLQI17.61.6). Twenty-four hours after catheter insertion, dialysates were collected for up to 12 h in lesional and non-lesional psoriasis skin every 1.5 h (for prostanoids), 3 h (for anaphylotoxins) and 4 h (for cytokines). Dialysates were analyzed by gas chromatography/mass spectrometry (negative ionization) for prostanoids: PGE2, PGF2α, 5- and 8-Iso-PGF2α, as well as for anaphylotoxins: C3a, C4a, C5a, and 17cytokines: IL1β, IL2, IL3, IL4, IL5, IL6, IL8, IL10, IL12p70, Fas-Ligand, TNFα, INFγ, Eotoxin, Rantes, IP10, GMCSF, MCP-1 using a cytometric multiplex bead array. We analyzed the difference of all mediators comparing lesional and non-lesional psoriasis skin in all patients and further divided into two subgroups: chronic plaques psoriasis with stable disease ($n = 9$, PASI 9.61.2;DLQI 15.30.6) and with acute exacerbation ($n = 5$, 18.43;DLQI 21.62.4).

Results: Cutaneous microdialysis was well tolerated in all patients over the time course of 36 h. In average, all mediators except MCP-1 showed higher values in lesional compared to non-lesional psoriasis skin. Significant differences we redetected for Prostanoids PGE2, PGF2α, 5- and 8-Iso-PGF2α, for anaphylotoxins C4a and for cytokines TNFα and Fas-Ligand. Whereas in patients with stable disease further significant differences between lesional and non-lesional skin could be observed for anaphylotoxins C5a, cytokines IL1β and IL8, no significant differences were detectable in patients with exacerbation, indicating high levels of inflammatory signals in non-lesional skin.

Conclusion: Cutaneous microdialysis is an interesting method to follow different levels of inflammatory responses *in vivo*. The results indicate that in case of acute exacerbation, the inflammatory response in non-lesional skin simulates the condition within a plaque.

P165
Altered migration of E/L-Selectin expressing DC – a monitoring challenge

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The Erlangen Dermatology Department is conducting a phase I/II trial (DERMA-ER-DC 06) with sequential adaptive design in advanced stage IV melanoma patients using autologous dendritic cells and specific RNA encoding MelanA, MAGE-3 and Survivin as source for antigen loading. A first

cohort of 17 patients was treated by up to 10 intradermal injections of 30 Mio RNA transfected DCs. To address the immunological impact of targeting DC to several lymph node regions and spleen, same RNA transfected DC were applied intravenously in Arm A of a 2nd cohort. Patients of Arm B received DC which additionally were transfected with RNA encoding a chimeric E/L-Selectin receptor to facilitate DC adherence to high-endothelial venules and thereby enable lymph node entry from the blood. Prior start of the 2nd cohort the functionality of E/L-S transfected human DC had been demonstrated by several *in vitro* assays.

The distribution of E/L-S transfected DC was analyzed in a first patient by whole body szintigraphy at various time points after i.v. application of 30 Mio 111 In-oxinate labelled DC transfected with E/L-S RNA only (no RNA encoding tumor antigens to avoid radioactive damage of antigen specific T cells). Thirty minutes after transfusion all cells were stuck in the lungs. Redistribution into liver and spleen started around 4 h posttransfusion and was completed at 19 h. A marked activity of cells was then also noted in bone marrow, especially of pelvis and spine. Even at d8 after cell transfusion spleen, liver and bone marrow were strongly labelled, however, we could not detect any activity within lymph nodes, not even by SPECT/CT performed at 70 h. Since the expected number of migrating DCs per LN is very small (approximately 5000 per LN), we analyzed LN, tumor tissue, blood and bone marrow of a patient 24 h after vaccination by flow cytometry and were able to clearly identify E/L-S DC in small numbers in the LN.

Now knowing that i.v. applied E/L-S DC do enter patients LN we have started further extensive immunomonitoring addressing homing receptor expression of induced T cell responses and also induction of humoral immune responses, to show whether optimized migratory behaviour of E/L-S does translate into superior immunopotency.

P166
Characterisation and functional analysis of T-cell responses in melanoma patients vaccinated with peptide-loaded dendritic cells

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T cell responses in melanoma patients vaccinated with autologous monocyte-derived dendritic cells (DC) loaded with peptides from different tumor-associated antigens (TAA) were characterized for their functional capacity.

To assign a certain functional capacity (cytolytic activity, cytokine production and degranulation upon restimulation) to specific T cell clones we chose a limiting-dilution based approach. Frozen aliquots of PBMC from five melanoma patients who had received four vaccinations were thawed, loaded with peptide and seeded in a 96 well plate followed by a 14-day culture. The cells were restimulated with autologous peptide-loaded PBMC at day 7. By splitting the cells two times during the 14-day culture four plates with identical clonal composition were obtained. Comparative analyses of each corresponding well were performed with the following assays:

1 Percentage of peptide-specific T cells, determined by MHC tetramer binding

2 Intracellular cytokine production (interferon-γ, interleukin 2, TNF-α) and degranulation (by CD107a mobilization) after antigenic stimulation

3 Cytolytic activity determined by a standard 51Cr-release assay

In all five patients vaccine-specific CD8+ T cells were detected after *in vitro* presensitation with peptide. Detected responses differed in magnitudes and overall functional capacity. In most cases a positive correlation between lytic activity and antigen-specificity (MHC tetramer positivity) was found. Furthermore the lytic activity correlated positively with certain cytokine profiles with a pronounced IFN-γ and TNF-α proportion and to a lower extend also with IL-2 and CD107a.

From our data can be concluded that vaccination with autologous monocyte-derived DCs loaded with TAA-derived peptides is capable to induce antigen-specific CD8+ T cells, with the potential to produce different immune-stimulatory cytokines and which show cell mediated cytotoxicity.

Further investigations of the induced T cells will be conducted to determine the breadth of the induced immune response by analysis of the different T cell clones and their affinities.

P167
Detection and sorting of melanoma cells circulating in patients' blood

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In many patients with solid tumors circulating cells demonstrating tumor characteristics have been identified in the peripheral blood. Such cells are usually referred to as circulating tumor cells (CTCs). Melanoma is notorious to metastasize via blood already at an early stage of the disease, but it may last many years before first metastases appear. We assume that CTCs may even have stem cell-like properties with the ability to remain quiescent for years before they begin to form metastases. To test these hypotheses not only detection but also isolation of the cells is necessary.

In the past, several procedures including immunocytology-, immunohistochemistry-based methods and RT-PCR have been used to detect tumor cells in the blood. However, since circulating tumor cells are very rare (1–5 cells in10 ml of peripheral blood), most detection methods are not sensitive enough. Furthermore, even if the cells have been detected by one or the other method, there was no way to further characterize the cells. We therefore set out to develop a suitable flow cytometry procedure. As first step we successfully enriched CTCs via the novel RosetteSep CD45 Depletion kit using 50 ml of whole blood from patients with extensive melanoma tumor burden. After a density gradient centrifugation the enriched cells were stained with melanoma specific markers. With our Cell sorter (BDARIA II, special order system) we were finally able to detect and sort CTCs (MCSP+MCAM+CD45-) ranging from 2 to 8 CTCs per 100 000 cells. This improved sensitivity allowed us to detect and sort melanoma cells in three groups of melanoma patients so far: (i) extensive tumor load (ii) bone marrow metastases (iii) elevated tumor markers S100 and MIA without detectable metastases even by PET.

In summary we describe an optimized procedure to detect and isolate CTCs out of whole blood from melanoma patients which, by allowing further tumor cell characterization such as drug resistance profiling, will hopefully become a future standard technology for patient individualized therapy.

P168
Disseminated granuloma annulare: successful anti-TNF-alpha treatment with adalimumab. A case series in six patients: clinical course and immunohistology

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Disseminated granuloma annulare, a necrobiotic granulomatous dermatitis degrading collagen, is notoriously difficult to treat. We report the successful treatment of six patients with adalimumab; moreover, we examined biopsies before and after 12 weeks' treatment by immunohistology since no such studies were reported previously.

In an open label study female patients aged 50–68 years were treated subcutaneously with an initial dose of adalimumab of 80 mg followed by 40 mg after 1 week and 40 mg every other week. Biopsies of skin lesions were taken before treatment and after 12 weeks and stained with H&E, anti-CD3, -CD4, -CD8, -CD54(ICAM-1), -HLA-DR, -CD1a, and -CD163 (activated macrophages) and evaluated microscopically. Skin lesions were also photographed at weeks 0 and 12.

We found a dramatic improvement of skin lesions in all patients studied. Before treatment there was an increase in all immunohistological markers, the most consistent strongest staining occurred with anti-CD54, -HLA-DR and -CD163. Clearing of skin lesions was accompanied with a varying decrease of CD3, CD4, CD8 and CD1a; however, there was a massive decrease of CD54, HLA-DR andCD163 after treatment in all patients.

We conclude that the clearing of disseminated granuloma annulare by adalimumab therapy is accompanied by an immense reduction of cellular inflammatory markers most notably ICAM-1, HLA class II and activated macrophages. This suggests that the necrobiotic inflammation in disseminated granuloma annulare is driven by TNF-alpha. Anti-TNF-alpha therapy should be considered as first choice for disseminated granuloma annulare.

P169
The significance of dendritic cells in the clearing of psoriatic skin lesions by Adalimumab

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We examined dermal dendritic cells (DCs) during a 12-week anti-TNF treatment with Adalimumab in 15 patients with psoriasis (five female, 10 male, aged 25–68 years). Clinical severity was determined by the psoriasis area and severity index (PASI), skin biopsies were taken from gluteal skin at weeks 0 from uninvolved and lesional skin and at week 12 from lesional skin. Skin sections were stained by H&E and monoclonal antibodies (visualized by biotin/streptavidin alkaline phosphatase) against HLA-DR (MHC class II), CD123 (IL3-Ra, plasmacytoid DCs), Langerin (CD207, cell surface receptor of Langerhans cells), CD11c (myeloid DCs), and CD83 (activated and fully mature DCs).

After 12 weeks, the PASI decreased from 21.9 ± 2.4 to 2.7 ± 0.9 (mean ± SE), the epidermal thickness (as measured by a micrometer grid, Leica Application Suite) from 358 ± 36 in lesional skin to 179 ± 14 μm (97 ± 4 in uninvolved skin at week 0). As counted microscopically, HLA-DR+ cells in epidermis and dermis did not differ between the various biopsies; neither did CD123+ cells between weeks 0 and 12 For Langer in we found a decreased number of epidermal DCs in involved epidermis at week 0 vs week 12 and uninvolved skin ($P < 0.001$; 2-tailed Wilcoxon test). By contrast, CD11c+ and CD83+ cells were significantly increased at week 0 in involved skin ($P < 0.01$) and returned to normal values at week 12 as in uninvolved skin at week 0.

Our findings indicate that MHC-class II+ and plasmacytoid DCs remain constant during anti-TNF treatment whereas Langerhans cells quickly increase in number in the epidermis. Moreover, myeloid (CD11c+) and activated (CD83+) DCs rapidly dwindle in the epidermis after successful therapy with Adalimumab. We conclude that the reverse shift of Langerhans cells and activated myeloid DCs during clearing of skin lesions is of pathogenic importance for psoriasis.

P170
Metallic nanoparticles activate the NLRP3 inflammasome and induce inflammation through the controlled release of both IL-1alpha and IL-1beta

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Nanoparticles are increasingly applied in various fields, such as biomedicine and electronics. By utilizing its opacifying effect, nano-TiO2 is frequently used in cosmetics and in sunscreens in particular. While TiO2 was considered to be biologically inert, an emerging amount of literature reports respiratory diseases in people exposed to the metall-oxide.

Here, we demonstrate that nano-SiO2 and nano-TiO2 activate the Nlrp3 inflammasome, leading to IL-1beta release. In addition, the regulated release of IL-1alpha is induced. Unlike other particulate Nlrp3 agonists, nano-TiO2-dependent-Nlrp3-activation leads IL-1alpha and IL-1beta secretion in non-phagocytic keratinocytes. *In vivo*, intraperitoneal injection of or pulmonary exposition to nano-TiO2 provokes an inflammatory response with the recruitment of neutrophils which is strongly dependent on the presence of IL-1-receptor-1 (IL-1R), the common receptor of both IL-1alpha and IL-1beta, and on IL-1alpha itself. Thus, the inflammation caused by nano-TiO2 *in vivo* is largely caused by the biological effect of IL-1alpha.

The current use of nano-TiO2 may present a health hazard due to its capacity to induce IL-1R-signaling, a situation reminiscent of inflammation provoked by asbestos exposure.

P171 (V12)
Interleukin (IL)-19, a novel component of the immunological cascades in psoriasis

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Psoriasis is a common chronic-inflammatory skin disease. Different immune cell-derived effector cytokines play key roles in the psoriasis pathogenesis. To expand our knowledge about these mediators, we quantified the expression of 30 different cytokines in the lesional skin from psoriasis patients. Surprisingly, the cytokine with the highest expression level and the greatest expressional increase compared to healthy control skin was Interleukin- (IL-)19. IL-19 is a novel member of the IL-10 – interferon family and can be produced by antigen-presenting and tissue cells. Since little is known about IL-19 production by skin tissue cells, we first investigated keratinocytes, melanocytes, dermal fibroblasts, dermal endothelial cells, and subcutaneous adipocytes for IL-19 production and found clear levels in activated keratinocytes. Detailed studies using different activation modes indicated IL-17 as a major inductor of this cytokine, although this cytokine had minimal if any effects on the expression of other IL-10 – interferon family members, including IL-22 and IL-26. Importantly, IL-22 strengthened the IL-17-induced IL-19 production. Apart from the high cutaneous expression, psoriatic patients showed strongly elevated IL-19 plasma levels, which correlated with the disease severity and the blood IL-22 levels. Finally, we performed a broad search for IL-19 effects on primary human keratinocytes. As deduced from gene chip-based analyses, IL-19 was surprisingly found to regulate the expression of a only few genes in these cells. These regulations suggested an IL-19-induced inhibition of the keratinocyte terminal differentiation (e.g. down-regulated expression of DSC1, FLG, and KRT10). This data shows that a novel keratinocyte mediator, being downstream of IL-17 and IL-22 in the pathogenetic cascades in psoriasis, may play a role in the epidermal alterations in that disease.

P172
Identification of *Staphylococcus aureus*'s adaptation capacity to antiseptics by microplate-laser-nephelometry

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Introduction: *Staphylococcus aureus* is one of the most important pathogens of nosocomial infections and is a common complication during the treatment of chronic wounds. 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. Hence, we have used an experimental system employing microplate-laser-nephelometry to test the adaptation capacity of *Staphylococcus aureus* to continued treatment with common antiseptics.
Material and Methods: Following antiseptics have been tested: polihexanide, chlorhexidine, PVP-iodine, silver nitrate, and octenidine. The antibiotic mupirocin was used as a reference. *Staphylococcus aureus* growth was investigated by microplate-laser-nephelometry and the respective IC50 concentrations of the antiseptics tested were determined. Subsequently, the microorganisms were repeatedly incubated with these IC50 concentrations for 100 days. Influence of the continued treatment was determined by calculation of the current IC50.
Results: A fast and dramatic increase in the IC50 of mupirocin was observed while the antiseptics showed a much lower potency to induce adaptation in *Staphylococcus aureus*. A slight rise of the IC50 was observed for polihexanide, octenidine and chlorhexidine over time. Furthermore, preliminary results for PVP-iodine showed aminor decrease of the IC50. In contrast, a distinct elevation of the IC50 was observed for silver nitrate.
Conclusions: Increasing use of antiseptics may result in bacteria that are less susceptible. As wound dressings with antiseptics are more and more utilized in the treatment of critical colonized or infected chronic wounds, it is of interest to determine the risk of triggering formation of resistant microbes. Employing microplate-laser-nephelometry it could be shown that commonly used antiseptics have a low potency to induce adaptation in *Staphylococcus aureus*. In the present study only the IC50 concentration for silver nitrate was found to increase with repeated treatment of *S. aureus*.

P173
ITS-sequencing in diagnosing cutaneous alternariosis – a case report

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Cutaneous infections with Alternaria species are rare and mostly diagnosed in immuno-compromised patients. Patients present with ulcers, nodes with hemorrhagic crusts or verruciform eczema-like skin lesions. Alternaria species are mostly dark pigmented featuring characteristic macroconidia.
We present the case of a 65 years old female renal transplant recipient who developed a 20 × 10 cm plaque at the left forearm without epidermal involvement such as scaling or crust formation. The patient was immuno-suppressed with tacrolimus, mycophenolat mofetil, and prednisolone.
Mycosis was diagnosed by histological and subsequent mycological examination which revealed non-pigmented mycelium without any macroconidia (Sabouraud glucose agar, Kimmig agar). The species or genus could not be identified using morphological criteria. ITS sequencing revealed Alternaria infectoria as pathogen. Later on, specific morphological features could be generated by using oatmeal agar. At this time point the antimycotic therapy had run for 2 weeks using the molecular biological results.
Initially, the patient was treated with voriconazole but developed renal failure due to elevation of the tacrolimus serum concentration generally induced by azoles. Moreover, the lesions did not improve. Therapy with caspofungin caused a reduction of inflammation while renal function recovered. After 7 months the infection was healed up.

P174
Strain differentiation of *Trichophyton verrucosum* using RAPD PCR (randomly amplified polymorphic DNA PCR)

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Until now, genetic strain typing of *Trichophyton verrucosum* for proving similarity of isolates is very difficult. Since the internal transcribed spacer is a highly conserved DNA region, its sequencing is not suitable for comparing different strains. A useful method for characterization of *Trichophyton verrucosum* strains is the randomly amplified polymorphic DNA (RAPD-) PCR.
We report a case of occupational *Trichophyton (T.) verrucosum* tinea corporis after needle stick injury with an attenuated live vaccine against cattle ringworm (Bovilis®Ringvac, Intervet Schering - Plough, Boxmeer, Netherlands). At the injection site, the patient developed a solitary itching and scaly erythematous plaque, measuring 2 × 3 cm. From the skin lesion, *T. verrucosum* was isolated showing its typical morphologic properties. On Sabouraud dextrose agar, the fungus displayed an elevated, orange pigmented mycelium and numerous chlamydospores as well as arthrospores. On Dermasel® agar, the thallus appear red greyish-white with submerse growth and a verrucous surface. The microscopic picture was characterised by a high number of macroconidia.
The cultivated commercial vaccine strain (Bovilis® Ringvac) showed a totally different phenotype with a white fluffy mycelium questioning whether the needle stick injury was really the mode of infection.
For RAPD PCR the primer pairs AB2-08, AB2-15, AB2-20, and AB2-02 (Hajdch M. etal., Folia Biol (Praha) 1999; 45: 151) were used. While AB2-15, AB2-20, and AB2-02 could not differentiate the isolate from different vaccine and wild type strains, AB2-08 produced a specific PCR fragment which was only found in our clinical isolate and the commercial vaccine strains Bovilis® Ringvac and LTF130 supporting our hypothesis of transmission and the occupational background of infection.

P175
A PBMC-transfer model to analyze human cutaneous leishmaniasis – suitability analysis of various immunodeficient mice

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Immunodeficient mice are used as recipients of human peripheral blood mononuclear cells (PBMC) for *in vivo* analyses of human immune functions. In the present study, we intended to establish a humanized mouse model for cutaneous leishmaniasis. First, we tested the suitability of the adult immunodeficient mouse strains NOD-Scid, NOD-Scid gamma (c)-/-, NOD-Scid-tg (HLA-A2.1) and H2b C57BL/6-likeRag2-/-gamma (c)-/- mice and transferred 50 × 106 PBMC i.p. from healthy volunteers. This was followed by intradermal infection with 1000 metacyclic L. major promastigotes into ears mimicking natural parasite transmission. Corresponding to human disease, all mice developed lesions at the inoculation site starting from week 3–4 post infection (p.i.). In case of NOD-Scid mice transgenic for the human MHCclass-I molecule HLA-A2.1 [NOD-Scid-tg (HLA-A2.1)] and H2b Rag2-/-gamma (c)-/-mice, we observed significantly increased lesion volumes after PBMC transfer compared to L. major infection alone (various time points). NOD-Scid gamma (c)-/-mice showed slight, but not significant increases in lesion volumes. However, significantly larger lesions were found in NOD-Scid mice when PBMC were transferred not only on day 0, but additionally in week 3 and week 6 p.i. (10 × 106 and 5 × 106 PBMC, respectively). Flow cytometric analysis of lesional leukocytes isolated from infected NOD-Scid ears (week 9 p.i.) showed a recruitment of up to 17% human CD45+ cells to the site of infection, mainly consisting of CD3+ T cells. The infiltrate was further composed of host CD11c+ DCs, F4/80+ macrophages and 7-4+ neutrophils. Further analysis showed that spleen and draining lymph nodes also harbored human CD45+ cells (CD4+ and CD8+ T cells, CD11c+ DCs, week 9 p.i.), but until now we were not able to detect release of antigen-specific human cytokines in these organs (NOD-Scid/ NOD-Scid-tg (HLA-A2.1)). Follow-up experiments using modified mouse strain NOD-Scid-tg (HLA-DQ) will show if this is due to lack of human MHC class-II-dependent antigen presentation. Interestingly, PBMC transfer triggered a xenogeneic GvHD in many cases, identified by dramatic weight loss, increased liver enzyme GPT levels and often subsequent death. This was the case not only for strains lacking NK cells (C57BL/6-like Rag2-/-gamma (c)-/-, NOD-Scid gamma (c)-/-), but also for adult NOD-Scid-tg (HLA-A2.1) and – unlike in prior studies – NOD-Scid mice. Future experiments will have to address how activation of human regulatory T cells or the use of alternative mouse strains (murine MHC class-I and/or class-II deficient NOD-Scid) will help to limit this effect and to establish a PBMC-transfer model for the analysis of human cutaneous leishmaniasis.

P176 (V18)
Antigen-loaded skin migratory Langerin+ DC induce regulatory T cells during Leishmania major infection

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Defense mechanisms against pathogens are exerted by different skin-derived dendritic cell (DC) subsets, including dermal DC (dDC) and Langerhans cells (LC). Previously, we demonstrated that Langerin+ DC are important for development of protective immunity against L. major by using an inducible *in vivo* ablation system, that is knock-in mice expressing a diphtheria toxin (DT) receptor (DTR) under the control of the langerin promoter. In the present study, we wanted to elucidate the mechanism and, in particular, which Langerin+ DC subset (dDC or LC) is responsible for this effect. Upon physiological low dose infection with L. major (1000 parasites), mice selectively depleted of LC (timed depletion protocol) developed significantly smaller ear lesions which correlated with reduced numbers of lesional CD4+ Foxp3+ regulatory T cells (Treg) as compared to control mice. In addition, C57BL/6 donor bone marrow (BM) was used to reconstitute lethally irradiated Langerin-DTR host mice. Radioresistant epidermal LC remain of host (Langerin-DTR) origin, whereas all other DC subtypes are radiosensitive and subsequently replaced by donor-derived cells. Again, DT-induced depletion of LC only led to significantly reduced lesions. In contrast, generation of reciprocal BM chimeric mice (donor: Langerin-DTR, host:C57BL/6) allows for the selective depletion of Langerin+ dDC, which had no beneficial effect for disease development. To study the suppressive capacity of Langerin+ DC in more detail, DC were isolated from lymph nodes of Langerin-DTR mice either treated with DT (Langerin+ DC depleted) or PBS (including Langerin+ DC). These two DC populations were pulsed with soluble Leishmania antigen and co-cultured with CD4+ CD25neg T cells. Induction of CD4+ CD25+ Foxp3+ T cells was significantly increased when the antigen presenting DC comprised Langerin+ DC as compared to those lacking Langerin+ cells. In conclusion, Langerin+ LC strongly contribute to induction of L. major-specific Treg and thus, vaccination strategies should aim to circumvent targeting LC.

P177
Characterization of Leishmania-derived CD8+ T cell epitopes by a combination of proteome analysis, epitope prediction followed by *in vitro* and subsequent *in vivo* analysis

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Healing of Leishmania infection is based on Th1/Tc1 immunity, since IFN-gamma secretion of both CD4+ and CD8+ cells plays a critical role for macrophage-dependent parasite killing. No effective vaccine candidates against this important human pathogen exist and only a single CD4+ T cell epitope (withTh2-predominance) has been characterized so far. To identify possible immunodominant CD8+ epitopes from the total proteome of the parasite, an epitope prediction approach was used. The most abundant proteins of both life forms of L. major, the infectious stage promastigote and the intracellular amastigotes, were identified by mass spectrometry. Subsequently, epitopes from these life form-specific proteins were predicted using the epitope prediction algorithm SYFPEITHI (www.syfpeithi.de). From these peptides, 300 were chosen based on their predicted immunoreactivity. To confirm this potential, a stability-assay was performed, in which the binding of the peptides to MHC-class I molecules was assessed. As a next step, all 300 peptides were tested in *in vitro* experiments using dendritic cells (DC) fromC57BL/6 mice as antigen-presenting cells. Peptides and immature DC were co-cultured with primed CD8+ T cells isolated from L. major-infected C57BL/6 mice, as they play a major role during parasite clearance. After 48 h, the supernatant was analysed for release of IFN-gamma and IL-4 from CD8+ T cells. About 20 out of 300 peptides were identified to be possible peptide candidates with a critical role for protective immunity against L. major in C57BL/6 mice, as these peptides induced CD8+ T cells to secrete high amounts of IFN-gamma and low levels of IL-4. The majority of these peptides are derived from a protein pool common to both parasite life forms, but some were amastigote- or promastigote-specific. In a next step, these epitopes will be tested for their potential to induce protective immunity *in vivo*. In summary, identification of novel CD8+ (and CD4+) T cell epitopes would (i) allow for detailed analysis of T cell development in infections with the parasite using e.g. the tetramer-technology and (ii) aid the development of a vaccine against this important human pathogen.

P178
Identification of clinical fungi by MALDI-TOF MS: how to deal with growth-dependent variability in peak patterns

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The identification of microorganisms by MALDI-TOF MS is about to replace biochemical identification procedures for routine diagnostics. While the mass spectral identification of most bacteria is straightforward, the identification of fungi with whole cell MALDI-TOF MS is more challenging for several reasons. Most importantly, the peak pattern of an individual isolate can change dramatically in dependence of incubation time and medium composition. Especially the transition from non-sporulating to sporulating mycelium generally results in marked differences in mass fingerprints.
One option to overcome this difficulty is to strictly standardize the cultivation conditions of reference and sample isolates. This can, however, be rather impractical due to differences in growth behaviour, particular medium demands, and handling requirements of individual isolates. Another strategy is to obtain reference data from well characterized isolates for different growth conditions. The latter strategy is followed for the Spectral Archive and Microbial Identification System (SARAMIS) by the acquisition of whole cell mass spectra of reference isolates grown on a variety of solid media and at

different incubation times. Generally, reference isolates are incubated on three different media and mycelium samples are taken after three different incubation times. By this 3 × 3 approach the variability of mass fingerprints of individual isolates is largely captured and the mass spectra are deposited in the reference database. When multiple isolates of a species are contained in the database, the corresponding data were used to compute SuperSpectra for fully automated identification.
SARAMIS allows the rapid, automated identification of most clinically relevant fungi by direct on-target smear preparation of fresh mycelium taken from agar plates. Examples will be presented for the identification of dermatophytes, yeasts, Aspergillus sp., and Fusarium sp.

P179
Mucosal protection against *C. albicans* infection by cathelicidin is induced by aTLR4 mediated crosstalk between PMNs and epithelial cells

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Immune responsiveness to many pathogens depends on innate recognition molecules known as pattern recognition receptors (PRRs) e.g. Toll-like receptors (TLRs). Infection of a three-dimensional organotypic oral epithelial model (oral RHE) with *C. albicans* suppressed TLR4 signalling despite clear evidence of mucosal injury and pro-inflammatory cytokine induction. Integration of polymorphonuclear leukocytes (PMNs) mediated upregulation of epithelial TLR4 and concomitant protection against fungal infection, which was independent of PMN/epithelial cell-cell contact. Candida invasion and cell injury could be restored by blocking TLR4 signalling using antibodies or RNA interference. Antibody neutralization studies demonstrated that the TLR4 mediated protective phenotype was associated with a pro-inflammatory cytokine release from PMNs, especially TNF, indicating an important role of this cytokine in the host defence against mucosal Candida infections. Given that the release of antimicrobial mediators by PMNs and epithelial cells might play a crucial role in the protective effect against *C. albicans*, we continued to characterize the PMN-dependent TLR4-mediated protection mechanism. The addition of PMNs to the Candida infected oral RHE model not only strongly upregulates epithelial TLR4 expression, but also the release of cathelicidin (LL-37) by the PMNs, which correlated directly with protection. The protective effect of LL-37 was abolished by the addition of TLR4-specific neutralizing antibodies andTLR4 'knockdown' (RNAi), demonstrating the direct role of LL-37 in the protective process. We confirmed the protective role of LL-37 by exogenous addition, which reduced *C. albicans* induced cell damage in the absence of PMNs. In summary, we were able to demonstrate that three-way immunological cross-talk between *C. albicans*, oral epithelium and PMNs results in the PMN-mediated up regulation of epithelial TLR4, which is directly responsible for protecting the mucosal surface from fungal invasion and cell injury by the secretion of LL-37 via PMNs.

P180
Interplay of *S. aureus* derived TLR2-ligands and IL-4R activation results in aggravation of atopic dermatitis inflammation

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Atopic dermatitis (AD) is a chronic inflammatory skin disease induced by infiltrating Th cells. Th2 cells dominate acute flares of AD as seen in atopy patch test lesions. Chronic AD lesions are often indistinguishable from other forms of eczema and co-dominated by Th1 cells. The mechanisms underlying this change of inflammatory pattern remained elusive. Colonization with *Staphylococcus aureus*, a gram-positive bacteria providing potent TLR2 ligands, is seen in >90% of AD patients. However, the impact of TLR2 ligands on AD inflammation is still unclear. Therefore we established a model for acute AD inflammation by adoptively transferring and activating OVA-specific Th2 cells in the skin of naive mice, in which the increase of ear thickness correlates with antigen-specific inflammation. While Th2 cells or OVA alone only lead to minor changes, Th2 cells plus OVA provoked inflammation and strong ear swelling after 24 h. Addressing the influence of pathogen associated molecular pattern (PAMP) we included into our experimental set up the *S. aureus* cell wall component lipoteichoic acid (LTA) or the synthetic lipoprotein Pam2Cys, both of themTLR2 ligands. Interestingly, LTA or Pam2Cys provoked prolonged and increased dermatitis, a pattern very similar to the OVA-specific dermatitis following transfer of Th1 cells. Cross-over experiments with TLR2-deficient mice and Th cells revealed that TLR2 on accessory cells but not on T cells is responsible for TLR2 mediated exacerbation of cutaneous inflammation. In addition, we could show that different TLR2 ligands stimulate dendritic cells (DC) to produce Th1 inducing IL-12p70 as well as IL-10. However, IL-10 is completely down regulated by IL-4 co-stimulation. This change of the IL-10/IL-12 ratio was observed *in vitro* and *in vivo*. Moreover, in our mouse model deficiency of IL-10 amplified inflammation while substitution with IL-10 neutralizes the IL-4/TLR-2 provoked enhanced dermatitis. Thus, we identified IL-10 as key regulator of TLR2 mediated inflammation. These data indicate that *S. aureus* derived TLR2 ligands shift Th2 cell dominated cutaneous inflammation towards chronic and persistent dermatitis through a concerted activation of TLR2 and IL-4R.

P181
Commensals amplify the innate immune response to pathogens in human skin

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Little is known about the impact of signals delivered by commensals on skin barrier function and the innate skin innate immune response towards pathogens. We show that commensal and pathogenic staphylococci differ in their ability to induce expression of antimicrobial peptides/proteins (AMPs) and activate different signaling pathways in human primary keratinocytes. Whereas secreted factors of *S. epidermidis* induce expression of the AMPs HBD-3 and RNase7 in primary human keratinocytes via TLR-2, EGFR- and NFkB-activation, those of *S. aureus* activate the MAPK- and PI3K/AKT signaling pathways and suppress NFB activation by upregulation of IkB. Interestingly, commensal staphylococci are able to amplify the innate immune response of human keratinocytes to pathogens by increased induction of AMP expression and abrogation of NFB suppression suggesting that the two activation pathways can act in a synergistic way. Two established model systems are available for studying the functional consequences of commensal induced immune conditioning in a more physiological context. First, the reconstituted human epithelial model, which has been already successfully used for analysis of the protective effect of *Lactobacillus rhamnosus* GG against *C. albicans*, and which can be supplemented with immune cells. Second, we established an *in vivo* skin colonization model in mice using epicutaneous inoculation of bacteria on tape stripped skin of C57BL/6 mice. Using these model systems we can study the effects of commensal microorganisms on pathogen infection of human and murine skin *in vitro* and *in vivo* in a physiological context.

P182
Induction of mouse beta-defensins in the skin of lipoxygenase (ALOXE3 andALOX12B) deficient mice with an ichthyosis-like phenotype

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Autosomal-recessive congenital ichthyosis is a heterogeneous group of hereditary keratinization disorders characterized by intense scaling of the whole integument, disturbed epidermal differentiation, and a disturbed skin barrier function. Mutations inALOXE3 and ALOX12B genes, which code for two different epidermal lipoxygenases preferentially synthesized in the skin, were found in patients with ichthyosiform erythroderma. We recently described induction of mouse beta-defensins after mechanically and metabolically disturbed skin barrier function. Beta-defensins are known as antimicrobial peptides of the innate immune system and are thought to protect against invading microorganisms. As mice with mutations in the ALOXE3 andALOX12B genes show an ichthychosis like phenotype with an impairment skin barrier function, we asked whether the expression of beta-defensins is induced in these mice. Flank skin samples from ALOXE3 and ALOX12B deficient mice were analyzed for expression of mouse beta-defensin-1, -3, and -14 mRNA and protein expression. Expression of mBDs was differently altered in the knockout mice. mBD-1 expression was only slightly induced in the skin of ALOX12B-/-, while there was no change in the skin of ALOE3-/- mice. Significant induction was found for mBD-3 a in both types of knockout mice. Expression levels of mBD-14 was slightly (not significantly) induced in both deficient mouse types. The intensity of mBD-3 and mBD-14 induction was much lower than previously found after mechanically and metabolically skin barrier disruption. In summary, we found partially induction of mBD-1, -3, and -14 expression in the skin of ALOXE3 and ALOX12B deficient mice. The increase in beta-defensin expression may subside for the loss of skin barrier function in mice with an ichthyosis like-like phenotype.

P183
Involvement of the transcription factor aryl hydrocarbon receptor in Leishmania major infection of macrophages

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In experimental L. major infection a Th1 response in C57BL/6 mice results in resistance while a Th2 response is associated with susceptibility in BALB/c mice. Macrophages are the most important host cells for L. major. The development of Th1/Th2 immune responses is driven by the early cytokine milieu in the infected tissue. Resident skin macrophages are among the first cells capable of cytokine secretion to come into contact with L. major, yet there are only limited data on the response of skin macrophages to L. major. In order to identify factors in the response to L. major which might ultimately influence disease outcome, skin macrophages from C57BL/6 mice were obtained and incubated with 5 metacyclical Leishmaniamajor/macrophage for 4 h. Microarray analysis showed significant upregulation of pro-inflammatory cytokines. Up-regulation of selected genes (e.g. TNFα, IL-1β, CCL4, CXCL1) was confirmed at the RNA and protein level. Also, the transcription factor aryl hydrocarbon receptor (AhR) was upregulated in C57BL/6 mice upon infection with L. major as early as 4 h.
The AhR is best known as a dioxin receptor and plays an important role in xenobiotic metabolism. Moreover, it was recently identified as modulator of Th17-celldevelopment and LPS induced macrophage activation.
In order to see if AhR is involved in (up) regulation of these cytokines we added AhR-antagonist (CH-223191, 30 nM) for 24 h to infected macrophages. This resulted in a reduction in mRNA for IL-1β, Cox-2, TNFα and CCL4 and lower amounts of TNFα and Mip-1β protein in the supernatants. Also, intracellular staining revealed a reduction in Cox-2 protein accompanied by reduced secretion of prostaglandin E2. Consistent with the fact that AhR has been shown to stimulate apoptosis we found that AhR antagonization enhanced the antiapoptotic effect of L. major infection in M-CSF starvation-induced apoptosis. AhR could potentially affect macrophage reaction to L. major infection via its known interaction with the Nf-κB pathway, but also a variety of other interaction partners.
Thus, we identified the transcription factor AhR as an important mediator of the response to L. major in macrophages. This makes AhR a possible target for treatment of cutaneous Leishmaniasis, especially so because the AhR can be easily manipulated with a structurally and functionally diverse range of ligands.

P184
Murine skin infection with *Staphylococcus aureus* is decided by both early mechanisms of innate immunity and T cell response in specific immunity

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Staphylococcus aureus (*S. aureus*) is the major human pathogen causing a diversity of skin infections. Once it overcomes the epithelial barrier it either remains locally controlled or spreads in the dermis causing soft tissue infection. We hypothesized that these different courses depend not only on its virulence factors, but also on genetically determined resistance of the host.
The objective of this study was to elaborate host factors which influence different outcomes of skin infection with *S. aureus*. Different inbred strains of mice were inoculated subcutaneously into the hind footpad with *S. aureus* strain SH1000. Swelling of footpads and visceral dissemination of *S. aureus* served as parameters for severity of infection. Cellular and humoral inflammatory reaction as well as antigen-specific T cell responses were analysed for differences in natural resistance.
We found that C57BL/6 mice are more susceptible than BALB/c. This higher susceptibility was reflected by significantly higher footpad swelling and increased dissemination of bacteria into inguinal lymph nodes and kidneys. Susceptibility in BALB/c mice correlated with significantly lower influx of polymorphonuclear leukocytes (PMN), reflected by lower activity of myeloperoxidase (MPO), and higher secretion of CXCL-2 which had been shown to increase intracellular survival of *S. aureus* in PMN. Elimination of PMN leads to fatal disease. Consistent with the high relevance of a PMN-rich innate immune response we found that molecules promoting inflammation and influx of leukocytes, e.g. Mrp8/14 and IL-6 were not only highly up-regulated, but also mandatory since infected Mrp8/14- and IL-6-deficient mice showed high susceptibility.
Since *S. aureus* infection persisted more than 2 weeks, we wondered if specific immune response also is relevant for control of infection. Remarkably, we not only found a *S. aureus* -specific T cell response, but also a correlation of resistance in BALB/c mice with a Th2 cell response and of susceptibility in C57BL/6 mice with aTh1 cell response.
Thus, we revealed genetically determined differences which decide about resistance to skin infection with *S. aureus* they include a higher activity of PMN as part of innate immune response and a *S. aureus*-specific Th2 response.

P185
Extracellular matrix components in skin barrier function

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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 (α1-α5), 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 into the five domains LG1 – LG5. LG1-LG3 modules are connected toLG4-LG5 by a linker domain, which is known to be sensitive to proteolytic processing. Interestingly, the LG4-5 fragment has been shown to be excised from the laminin α3(LAMA3), α4 (LAMA4) and α5 (LAMA5) chain in various tissues. However, the functional role of this fragment has remained almost unknown to date.
In this study we show that peptides derived from the human laminin alpha 3, 4, 5 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 expression of LAMA3, LAMA4 and LAMA5 in human keratinocytes and fibroblasts was upregulated by bacterial infection. Finally, LG4-peptides show chemotactic activity for various PBMCs.
In summary, our data suggest that components of the extracellular matrix might playa role in the innate immune response of epithelia by protecting the respective tissue from invading pathogens.

P186
Characterization of *Staphylococcus aureus* colonization among patients with atopic dermatitis in northern Germany

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Staphylococcus aureus is one of the most important human pathogens and methicillin-resistant *S. aureus* (MRSA) strains became a global burden to the health system. In patients with atopic dermatitis (AD), *S. aureus* colonization is high and positively correlates with the severity of their eczema. Despite this observation, deep skin infections as well as systemic infections occur rarely in AD patients. Moreover, little is known about the rate of MRSA in AD patients. In order to investigate this phenomenon, we initiated a prospective study to characterize and genotype the *S. aureus* strains derived from the anterior nares and from skin among AD patients.
Bacterial swaps of lesional, non-lesinal skin and from the nasal area were taken from44 AD patients in our outpatient clinic and from 16 control persons. Bacteria were cultured and identified using standard methods. Ambiguous identification results were verified by 16S rRNA gene sequencing. All isolates were genotyped by sequence typing of the variable region of the staphylococcal spa gene. Virulence genes of interest were investigated by PCR methods.
Forty-four patients were included so far in the study. *S. aureus* was detected in 31 patients (70.4%) showing a broad range of different spa types except spa type t091(equivalent to MLST ST7) found in both AD patients and the control group. None of the detected *S. aureus* strains was methicillin-resistant. All were tested Panton-Valentine leukocidin-negative. For lesional *S. aureus* isolates, the possession of pyrogenic superantigen genes was variable comprising isolates without any of the toxin genes tested and those harbouring SEB-, SEC-, and SEG/SEI-encoding genes. The most common agr type of lesional *S. aureus* isolates was agr-1 followed byagr-2. In five out of 16 control persons, *S. aureus* was detectable, all methicillin-sensitive.
Our preliminary results suggest that MRSA occurs rarely in AD patients. It will be of future interest to follow these patients and investigate if the colonization with specific strains will change over time.

P187
The effects of staphylococcal lipoteichoic acid on skin inflammation depend on the strength of stimuli

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Organs at the interface such as the skin contact microbes continuously and microbial pathogen associated molecular pattern (PAMP) contribute to signals which determine cutaneous responses. *Staphylococcus aureus* is a known trigger for inflammatory skin diseases, especially atopic dermatitis, whereas *Staphylococcus epidermidis* is part of the normal skin microflora. Both species set free enormous amounts of the cell wall component lipoteichoic acid (LTA) and it was the aim of this study to reveal the role of LTA for cutaneous immune responses. Hence, we established a contact hypersensitivity (CHS) mouse model, the fluorescein isothiocyanate (FITC) CHS, which mimics atopic dermatitis with Th2 cells and high IgE levels, for the study of effects of LTA. Presence of LTA during repetitive contacts with FITC enhanced FITC-specific skin inflammation and induced Th1-immunity demonstrating a role of LTA as PAMP. On the contrary, limited application of LTA during initial contacts to FITC significantly suppressed lesional T cell cytokine expression such as IL-4 and IFN-γ. The proliferation of the T cells from the draining lymph nodes of the LTA-treated skin was also highly reduced. In order to understand the underlying mechanisms of this effect, CD4+ T cell activation was analyzed *in vitro* in the absence of other cell types by polyclonal activation. Surprisingly, whereas otherTLR2 ligands acted as T cell costimulators, LTA treatment led to significant suppression of T cell proliferation. Those T cells were still viable and did not show more Annexin V staining than their control group. To summarize, there are two opposing biological functions of LTA: (i) Enhancement of severe or ongoing inflammation through induction of Th1 immunity as clinically observed in chronic atopic dermatitis and *Staphylococcus aureus* colonization. (ii) Suppression of mild inflammation by direct inhibition of T cell responses. This may be of great relevance as it indicates how on the one hand resident microflora such as *S. epidermidis* mediates tolerance and even stabilizes the cutaneous immunological barrier and how on the other hand this mechanism could be exploited by cutaneous microorganisms to evade effective immune responses.

P188
Papular-purpuric gloves and socks syndrome – an IgM-immune complex mediated disease entity?

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A previously healthy 42-years old caucasian woman was admitted to the ER with fever up to 39°C, severe malaise and massive itchy swelling of both hands and feet, with dark red discoloration of the skin that was strictly demarcated at the ankles and wrists. Besides obesity with a body mass index of 64 (normal 21–26), the clinical examination was inconspicuous and the patient denied the intake of any medication. Laboratory work up revealed elevated C reactive protein of 5 mg/dl, an erythrocyte sedimentation rate of 24/50 and slight elevation of liver enzymes (GPT 48 and GOT52). HbA1c was elevated to 73%, while bcc and renal parameters were normal. Under the primary diagnosis of an angioedema, the patient was first treated with Dimentiden i.v. Due to progression of the clinical symptoms, with petechia, confluent erythematous papules and vesicles, as well as new erythematous areas with central necrosis in the genital and oral mucosa, the patient was transferred to our department. At this time, Parvovirus B19-specific IgM and PCR as well as circulating IgM-immune complexes were positive, while Parvovirus B19-IgG was negative. Titers of other exanthema-related viruses only showed previous encounters. Histopathology showed an acanthotic epidermis with necrotic keratinocytes, intraepidermal cleft formation and subepidermal edema with a patchy inflammatory infiltrate of mixed leucocytes, nuclear dust and extravasated erythrocytes was observed. Based on these findings, the diagnosis of Papular-purpuric gloves and socks syndrome (PPGSS) was established. IgG seroconversion was observed 7 days after disease onset. Fever and skin lesions gradually resolved under treatment with paracetamol 500 mg 3× daily p.o., topical mometasone, and octenidin wrappings. Fourteen days after initial symptoms, the patient was dismissed in good condition without skin lesions. The observation of circulating IgM-immune complexes together with Parvovirus B19-specific IgM at the time point of first (vasculitic) skin lesions points to a pathogenic role for circulating IgM-immune complexes in PPGSS.

P189
Human papilloma virus (HPV) typing by DNA-sequencing in 49 patients with condylomata acuminata

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The incidence of condylomata acuminata (genital warts) is rising alarmingly. Potential risks of HPV infection include carcinogenesis. HPV types involving a low risk for carcinogenesis involve 6, 11, 57; HPV types 16 and 18 are associated with a high risk.
The diagnosis in 49 patients (42 male, seven female) of our department was confirmed by histopathology. In order to indentify HPV-variants we amplified the DNA by PCR using MY9/MY11 primers. Afterwards we sequenced the isolated DNA employing the Sanger-Method.
In 43 specimens we were able to isolate HPV and to identify five different HPV-types.79.1% were HPV-6 positive, 9.3% exhibited HPV-11 and 2.3% showed HPV-16.2.3% of the HPV-positive specimens were infected with HPV-83 and 2.3 were infected with HPV-57. 4.7% of the specimens were infected with multiple HPV which could not be processed with Sangers method.
We conclude that 90% of condylomata acuminata are associated with low-risk HPV variants for carcinogenesis, at least 2% are high risk variants. Our findings indicate that multivalent HPV vaccines including HPV types 6, 11 and 16 could decrease the incidence of genital warts.
The results are part of the doctoral dissertation of P. Beier.

P190
Pharmacokinetics of fumaric acid esters in portal vein blood of rats

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Dimethyl fumarate (DMF) is the essential ingredient of a registered, well established drug product (Fumaderm®, Biogen Idec, Ismanning, Germany) used for the systemic treatment of psoriasis. Recently, it was shown in a phase II clinical trial that DMF as monotherapy is beneficial in the treatment of relapsing-remitting multiple sclerosis.
The central question to be answered that will help to understand the mode of action of DMF is if DMF itself or one of its metabolites represents the active substance *in vivo*.
In previous pharmacokinetic studies with fumaric acid esters in humans plasma levels of DMF were always below the level of detection suggesting a complete degradation of DMF already in the small intestine. However, in a recent study our group described the presence of the mercapturic acid of DMF in urine of psoriasis patients after oral intake of Fumaderm® suggesting that DMF is absorbed into the presystemic circulation namely into the portal vein blood. As it is likely that DMF mediates its immunomodulating effects by reacting with intracellular glutathione (GSH) of immune cells in the portal vein blood we initiated a study in rats and asked if free DMF can be detected in blood of the portal vein after oral application of a DMF solution into the small intestine.
Before the DMF solution was applied a portal vein catheter was implanted and secured. Prior to dosing a first blood sample was taken from the portal vein. Thereafter, 20 mg/kg of DMF or control solution was added through a gauge placed in the small intestine. Blood samples were taken from the portal vein after 2, 5, 10, 15, 20, 30 and 60 min. Blood samples were placed in vials containing 4 mg/ml NaF as anticoagulant and inhibitor of esterases. After centrifugation plasma was stored at -80°C until analysis by HPLC.
The results show that the hydrolysis product of DMF, monomethyl fumarate (MMF), is already detectable 2 min after the application of DMF, but DMF could not be detected at any time point.
This finding points towards the importance of the reaction between glutathione and DMF under *in vivo* conditions. In further studies portal vein blood has to be analysed for GSH-adducts with DMF.

P191
Electron paramagnetic resonance study of nitroxide loaded invasomes - penetration and drug delivery *ex vivo* and *in vivo*

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Background: Various nanometer scaled transport systems are used in pharmaceutics and cosmetics for penetration enhancement and enhanced storage of drugs applied to the skin. Standard liposomes consisting of a rigid gel-state membrane were shown to be less effective penetration enhancers than liquid state ultra flexible vesicles with elastic membranes. Among these ultra flexible vesicles – invasomes consisting of phosphatidylcholine, ethanol and terpenes – were shown to be effective drug delivery systems. Different theories describe the penetration of liposomes. Several EPR measurements were performed on liposomes but no data are available for ultra flexible invasomes and no *in vivo* measurements using EPR technique were published in this context.
Objectives: In this study, the partitioning of the amphiphilic spin label TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) between membrane and aqueous phase has been studied by multi frequency EPR. Furthermore, TEMPO partitioning was monitored during invasome penetration into porcine skin using two EPR based analysis methods. Consequently, the TEMPO invasomes were applied on the forearms of human volunteers and degradation of TEMPO was measured.
Methods: Invasomes were prepared by the conventional mechanical dispersion method and TEMPO was applied to the lipid phase. Afterwards invasomes were measured at W, Q, X and L-band frequency as a prerequisite for simulation and determination of magnetic parameters with the EasySpin toolbox. By the use of the magnetic parameters simulation of TEMPO partitioning during invasome penetration into normal and barrier disturbed (20 tape strips) porcine skin was performed. As control all experiments were also performed using TEMPO in PBS/ethanol solution.
Results: Different penetration mechanisms of invasomes could be observed for normal and barrier disturbed skin, indicating that invasomes disorganise intercellular lipids. The fraction of TEMPO associated with the membrane phase was determined to 33% before application onto the skin. For normal skin it increased to 95% and for barrier disturbed skin to 50% during penetration. Invasomes applied *ex vivo* and *in vivo* were shown to stabilize TEMPO, while application of the free label in solution leads to a rapid degradation of the label due to reaction with antioxidative species.
Conclusion: Using magnetic parameters derived from high frequency measurements at W-band, the determination of partitioning in invasomes becomes feasible at low frequency L-band. Therefore, L-band EPR could be used *ex vivo* on porcine ear skin and, for the first time, *in vivo* on the forearm of healthy volunteers to monitor partitioning and penetration processes by spin label observation dur-

ing penetration into the skin. Invasomes were shown to be slow release depot systems. The results obtained *in vivo* are generally comparable with those obtained *ex vivo*.

P192
Role of dipeptidyl peptidase IV and related enzymes in the regulation of DNA synthesis of skin cells

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Dipeptidyl peptidase IV/CD26 is an ectoenzyme up-regulated in hyper proliferative skin diseases like psoriasis. Primary human keratinocytes, skin fibroblasts and sebocytes were previously described to express DPIV/CD26 on the cell surface. The non-selective inhibitors of DP IV-activity, Lys[Z(NO2)]-thiazolidide (LZNT) and-pyrrolidide (LZNP) suppress proliferation and cytokine production of these cells *in vitro*, suggesting this enzyme as a target for drug therapy of skin diseases like acne or psoriasis. The role of other DP IV-related enzymes like DP 8/9 in the regulation of cellular functions was hypothesized, because sitagliptin, a DP IV-selective inhibitor approved for diabetes therapy, lacked immunosuppressive activity. The aim of the present investigation was to clarify the role of DP IV activity and DP IV-related enzymes in the regulation of DNA synthesis of skin cells.

We studied expression of DP 8/9 in relation to DP IV by quantitative RT-PCR and investigated the dose-dependent and maximum suppression of Gly-Pro-pNA hydrolysis and [3H]-Thymidine incorporation in the presence of a DP8/9 inhibitor and the DP IV-selective inhibitor DP IV-I/6 compared to LZNT. Proliferation assays were performed to detect the suppressive capacity of various inhibitors of DP IV-like activities, among them the diabetes-approved inhibitors Sitagliptin, Saxagliptin andVildagliptin and newly developed inhibitors, which do not interact with the active site. As shown on activated T cells, these inhibitors bind to the central pore binding site of DP IV and induce a sterical inhibition of processing natural substrates.

We found that keratinocytes (HaCaT and NHEK) and SZ95 sebocytes significantly express DP8/9 on the mRNA-level. In all homogenized cell types, approximately 2/3 of DP IV-like activity can be suppressed by the DP8/9-inhibitor, which has no effect on DNA synthesis. Moreover, we observed that in NHEK keratinocytes, DP IV-I/6 suppresses 20.6% of enzyme activity and 55.4% of DNA synthesis, whereas in HaCaT keratinocytes neither enzyme activity nor proliferation are reduced. In SZ95 sebocytes, the DP IV-selective inhibitor has minor effects on enzyme activity (4.3%) and DNA-synthesis (36.20%). A combination of a DP IV and a DP8/9-specific inhibitor shows additive effects on suppression of enzyme activity, but no similar effect on proliferation, and is comparable to the effects seen with LZNT in all cell types. Other inhibitors (including the marketed drugs for diabetes therapy) did not suppress the proliferation of the skin cells, although they all inhibit soluble DP IV and some also DP8/9 at nanomolar concentrations. Most interestingly, the best effects on the skin cell proliferation were achieved by those inhibitors that bind to DP IV, but not at the active site.

Our data suggest that for the suppression of skin cell proliferation, the inhibitory capacity toward DP IV-activity or distinct purified DP IV-like enzymes is not crucial. With respect to membrane bound DP IV, it is highly probable that sterical inhibitory effects on the access of natural substrates and/or the induction of conformational enzyme structure changes are much more relevant. The most probable explanation is an alternative binding site at DP IV mediating the antiproliferative. These data support the novel model of cellular DP IV function developed recently by IMTM.

P193
A novel method to educate patients how to apply a sufficient amount of sunscreen aided by *in vivo* attenuated total reflection FT-IR spectroscopic imaging

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Fourier transform infrared (FT-IR) spectroscopic imaging with focal plane array detectors has proved a powerful technique for rapid visualisation of a huge number of different chemicals. It offers the possibility of combining spectral and spatial information. *In vivo* IR imaging is an important new field of application. In a feasibility study the application of this technique was described for the *in vivo* investigation and visualisation of different layer thickness of sunscreen products (0.5, 1 and 2 mg/cm) 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 which is used for the measurement of sun protection factors (SPFs) according to EU and other international standards.

In a consecutive experiment we could show that two applications in a 30 min interval resulted in quantities similar to this required amount. This approach offers a practical and comprehensive way for consumer to achieve a sufficient sunscreen application – just 'think twice'. With the resulting IR imaging pictures very demonstrative and convincing educational material is available for the first time. It can be used for patient training in order to show and convince them how important the right amount of applied sunscreen product is and how easily sufficient protection could be achieved.

It is essential to have effective protection against sunburn, photoageing and skin cancer especially for people with photodermatoses, photoallergies, drug-induced photosensitisation or immunosuppression.

P194
UVB triggers interleukin-1 beta release in human epidermal keratinocytes through AIM2

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The skin is the first barrier against environmental stresses such as UVB irradiation. UVB causes sunburn and triggers cutaneous inflammation by increasing interleukin-1beta (IL-1 beta) release. IL-1-beta subsequently activates the infiltration of inflammatory mononuclear cells. Recently, it was demonstrated that UVB irradiation causes a change in intracellular ion concentration with release of Ca2+ in human keratinocytes. This leads to the activation of the cysteine protease caspase-1 which is required for IL-1 beta secretion. In further studies it was shown that the NALP3 inflammasome is also involved in UVB induced caspase-1 and IL-1 beta activation. In the present study we confirm that UVB irradiation of skin biopsies obtained from healthy volunteers leads to increased IL-1 beta release. Also, the active form of caspase-1 (p20) was only found in cell lysates of irradiated skin. In RNAi knockdown experiments we observed that in cultured keratinocytes (NHEK) the inflammasome components ASC, NALP3 and caspase-1 control IL-1 beta release. Furthermore we observed that a novel inflammasome is also involved in the UVB induced IL-1 beta release: The recently discovered cytosolic protein absent in melanoma 2 (AIM2) senses cytosolic, double-stranded (ds) DNA and triggers inflammasome activation. In our experiments, AIM2 knockdown by siRNA strongly reduced UVB-triggered IL-1beta release in NHEK. As AIM2 is a cytosolic DNA receptor we investigated howAIM2 is activated in UVB irradiated cells. Physiologically DNA is present in the nucleus but not in the cytosol of eukaryotic cells. Still, PCR analyses revealed the presence of genomic DNA in the cytosol of keratinocytes upon UVB irradiation. Therefore, we suggest that UVB irradiation triggers the release of DNA from the nucleus to the cytosol in keratinocytes. Cytosolic DNA then binds to AIM2 and activates the inflammasome which leads to IL-1 beta activation and cutaneous inflammation.

P195
Inhibition of the effector phase of contact hypersensitivity in sensitized mice via *in vivo* induction of Foxp3+ regulatory T cells by ultraviolet radiation

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Ultraviolet radiation (UVR) induced regulatory T cells (UVR-Treg) inhibit the sensitization but not the elicitation of contact hypersensitivity (CHS) when injected i.v. because UVR-Treg express lymph node, but not skin homing receptors and thus migrate into the lymph nodes but not into the skin. It was shown that the homing receptor expression and the migration of UVR-Treg can be altered by tissue-specific antigen presenting cells both *in vitro* and *in vivo*. Mice sensitized against dinitrofluorobencene (DNFB) through the abdomen were exposed to UVR on the back followed by epicutaneous application of DNFB on the UVR-exposed skin to induce UVR-Treg. Since these cells migrate into the lymph nodes the effector phase of CHS is not suppressed. However, when UVR-Treg located in the lymph nodes were stimulated *in vivo* by antigen presenting cells from the epidermis via application of DNFB on the flank, UVR-Treg migrated into the skin and thus inhibit the elicitation of CHS in sensitized mice. The stimulation of UVR-Treg by antigen presenting cells from the skin is crucial, since the inhibitory effect was lost upon depletion of langerin-positive cells, as demonstrated in langerin diphtheria-toxin (DT) receptor knock in mice treated with DT. Treg induced *in vivo* by UVR express Foxp3 since the inhibitory effect was lost in mice expressing a DT receptor-enhanced green fluorescent protein fusion protein under the control of the foxp3 gene locus, allowing selective and efficient depletion of Foxp3+ Treg upon DT injection. Together, these data demonstrate that Treg can be induced *in vivo* by UVR in sensitized mice and that these cells can be altered in such a way that they inhibit the elicitation of immune reactions in sensitized individuals. This indicates a first *in vivo* strategy to utilize Treg not only for the prevention but also for the treatment of immune-mediated diseases.

P196
Impact of UVA radiation on UVB-induced apoptosis in normal human melanocytes

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It is generally accepted that chronic sun exposure is a risk factor for the development of non-melanoma skin cancer. In contrast, the exact correlation between melanoma and sun light is not clear. So far most investigators focused on the effects of particular solar spectra (mainly UVA or UVB). However, natural sunlight contains both UVA and UVB in various ranges which depend on daytime, season, altitude and latitude. Hence, we investigated the impact of the interplay between UVA and UVB on apoptosis and DNA damage in human melanocytes *in vitro*. Normal human melanocytes of different donors were pretreated with 20 J/cm of UVA-1 (340–400 nm) and 3 h later with 0.4 J/cm of UVB (290–320 nm). Twenty-four hours later the apoptotic rate was determined using a cell death detection ELISA. UVB-induced apoptosis was significantly reduced in cells pretreated with UVA-1 in comparison to melanocytes irradiated with UVB only. UVA-1 alone did not induce apoptosis. To assess the influence of UVA-1 on UVB-induced DNA damage which is the major molecular trigger for apoptosis, the amount of cyclobutane pyrimidine dimers (CPD) was evaluated 6 h after irradiation with 0.08 J/cm of UVB by South-Western dot-blot analysis using an antibody directed against CPD. Pretreatment of melanocytes withUVA-1 did not alter the amounts of CPD, indicating that UVA-1 does not influence DNA-repair. Since UVA-1 reduces UVB-induced apoptosis of melanocytes in a DNA damage-independent manner, other mechanisms like the alteration of the expression of apoptosis-related proteins may be involved. Elucidation of these mechanisms appears to be important since UVA-1 may support the survival of melanocytes carrying UVB-induced DNA damage and thereby contribute to melanoma genesis.

P197
Consequences of PUFA supplementation on the UV-response

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Supplementation with very long chain polyunsaturated fatty acids (PUFA) as are present in marine fish is widely regarded as healthy. Clinical data underline that consumption of marine fish (-oil) significantly improves cardiovascular, inflammatory and neurological parameters in human populations. Many of the positive effects of PUFA supplementation are attributed to the function of omega-3 fatty acids as precursors of (anti-inflammatory) signal mediators.

We propose that an entirely different mechanism may also contribute to desirable outcome of PUFA supplementation. Our recent work has dealt with the effect of oxidized phospholipids in the skin. We have shown that UVA- oxidation of polyunsaturated phospholipids induces a cell protective and anti-inflammatory response by activating the redox sensitive transcription factor Nrf2. If free PUFA are incorporated into the cell membrane phospholipids of skin cells and these are subjected to moderate oxidative stress, this might result in an overall protective, Nrf2 mediated response of the cells.

To test this concept, we supplemented dermal fibroblasts with saturated, mono, and polyunsaturated free fatty acids alone or in combinations that are found in fish oil and olive oil. Mass spectrometric analysis revealed that docosahexaenoic acid (DHA) is very efficiently incorporated into membrane lipids with a phosphocholine backbone. Upon UVA-irradiation, these lipids were susceptible to oxidative modification, as proposed. We investigated the cellular UV-response of PUFA supplemented versus control cells and cells supplemented with saturated fatty acids. PUFA supplemented cells showed a massively stronger induction of Heme oxygenase -1 and other Nrf2 dependent genes of the antioxidant response. Also, the PUFA supplemented and UVA-1 irradiated cells displayed a strongly reduced inflammatory response after chemical or TLR- induced inflammation.

Or data indicate that activation of the Nrf2 dependent antioxidant response might contribute to the cell protective and anti-inflammatory effects observed after supplementation with fish oil rich in polyunsaturated fatty acids.

P198
UVB-induced skin hemorrhage during thrombocytopenia depends on leukocyte recruitment

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In previous work concerning the role of platelets in vascular integrity during inflammation we observed that during thrombocytopenia UVB-irradiation causes skin hemorrhage (Purpura solaris) in mice.

To further characterize this phenomenon, thrombocytopenic C57BL/6 and BALB/c mice were exposed to increasing doses of UVB-radiation (210–1260 mJ/cm). The analysis of irradiated skin 0–24 h later showed a dose- and time-dependent but strain-independent manifestation of Purpura solaris. Moreover, UVB-irradiation induced a significant cutaneous influx of neutrophils that was observed in histological sections of skin biopsies and quantified by measuring the activity of the neutrophil-specific myeloperoxidase (MPO). The UVB-induced skin bleeding is strictly limited to the sites of irradiation and can be prevented by dose-reduction e.g. via topical application of sunscreen (SPF > 50).

As UVB-irradiation induces a rapid influx of leukocytes, their role was further investigated in UVB-induced skin hemorrhage under thrombocytopenic conditions. To this end, mice were treated with a neutrophil depleting antibody prior to UVB-radiation. Interestingly, under leukocytopenic conditions skin bleeding was virtually absent. These findings suggest that leukocyte extravasation is required in the development of Purpura solaris. The role of chemoattractant stimuli essential for leukocyte recruitment is matter of current investigation.

In summary, during the absence of platelets UVB irradiation induces Purpura solaris which can be prevented by inhibiting cutaneous leukocyte recruitment.

P199

Angiopoietin-2 stimulation induces αvβ3 integrin internalization and degradation

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Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2) have been identified as agonist and antagonist of the endothelial tyrosine kinase receptor Tie2. While Ang-1 induces phosphorylation, Akt activation and subsequent endothelial cell survival, Ang-2 interferes negatively and induces endothelial destabilization. Both, Ang-1 and Ang-2, have been demonstrated to induce junctional translocation of its receptor Tie2. To molecularly decipher the mechanisms of Ang-2-mediated endothelial cell destabilization, we examined the consequences of Ang-2-induced junctional Tie2 translocation. Immunofluorescent analyses revealed that both, Ang-1 and Ang-2, were capable to recruit β3 integrins into the interendothelial junctions. However, onlyAng-2 stimulation resulted in complex formation between Tie2 and αvβ3 integrin. Ang-2-induced complex formation of Tie2/αvβ3 integrin, included Focal Adhesion Kinase (FAK). FAK was getting phosphorylated in the FAT domain at Ser-910 which was followed by dissociation of the adapter proteins paxillin, p130Cas and talin. Theαvβ3 integrin was internalized, ubiquitinylated and gated towards lysosomes. Taken together, the experiments have unraveled Tie2/αvβ3 integrin association, integrin internalization and degradation as mechanistic consequence of endothelial Ang-2 stimulation.

P200

Inhibition of the PI3K-AKT signalling pathway to overcome therapy resistance in melanoma-derived brain metastasis

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Brain metastases occur in over 70% of patients with metastatic melanoma and are the most common cause of death. Current therapy options are neurosurgery, radiosurgery, whole brain radiation, chemotherapy and supportive care. The median survival time for melanoma patients with brain metastasis ranges from 0.7 to 5 months depending on age and performance status. Therefore, new therapy strategies are mandatory.

In metastatic melanoma, the RAF-MEK-ERK (MAPK) and the PI3K-AKT-mTOR (PI3K) signalling pathways are constitutively activated through multiple mechanisms. We investigated the effects of classical and new pharmacological RAF, MEK, PI3K and mTOR inhibitors on growth of brain-metastatic melanoma cell lines and cells directly isolated from melanoma brain metastases. Brain-metastatic melanoma cell lines and cells directly isolated from melanoma brain metastases were resistant towards selective BRAF, MEK and mTOR inhibitors, whereas PI3K inhibitors could achieve growth inhibition rates up to 80%.

A histochemical analysis showed that melanoma brain metastases of 10 patients were highly positive for activated AKT, whereas the surrounding tumor-free brain tissue was negative for activated AKT.

Together, these findings suggest that the activation of the PI3K-AKT signalling pathway is relevant for the survival and growth of melanoma cells in the brain parenchyma and that inhibition of this pathway may be a good strategy to overcome therapy resistance in melanoma brain metastasis.

P201

Temozolomide chemoresistance in prolonged melanoma treatment regimens: assessment of mechanisms

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The efficacy of temozolomide in melanoma treatment is low (response rate <20%) and may depend on the activity of O6-methylguanine DNA methyltransferase (MGMT) and mismatch repair. The identification of molecular resistance mechanisms would be a way to enhance temozolomide treatment efficacy. Moreover, such knowledge would help to identify biomarkers that predict treatment response allowing individualized therapies. We identified melanoma cell lines with different sensitivities to single versus prolonged clinical dosing regimens of temozolomide treatment and assessed a variety of potential resistance mechanisms using this model. Towards this end, mRNA expression and promoter methylation of O6-methylguanine-DNA-methyltransferase (MGMT) and essential mismatch repair genes (MLH1, MSH2) were measured. Cell cycle distribution, apoptosis/necrosis induction, O6-methyl guanine-adduct formation, and ABCB1 gene expression were assessed. KAII and LIBR cells were more sensitive to a prolonged, whereas MelA, MelB, and MelC cells were surprisingly more sensitive to a single dosing regimen. Only MelC expressed MGMT. Gene expression correlated well with promoter methylation. Temozolomide exposure did not alter mRNA expression. Different sensitivities to temozolomide were caused neither by delayed apoptosis induction due to early cell cycle arrest nor by O6-methylguanine-adduct formation and elimination or efflux transporter expression. We conclude that MGMT expression is a relevant factor of temozolomide chemoresistance that can, at least in part, explain the low melanoma treatment efficacy. Considering individualized temozolomide treatment regimens seems worthwhile.

P202 (V04)

The HSP70 inhibitor MAL3-101 affects merkel cell carcinoma *in vitro* and *in vivo*

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Merkel cell carcinoma (MCC) is a rare but highly aggressive skin cancer which primarily affects elderly or immunocompromised patients. Current therapies are only of limited efficacy. Thus, the discovery that in about 80% of MCC cases the Merkel cell polyomavirus (MCV) is present opens new therapeutic options, especially since we recently demonstrated that MCV positive MCC cells depend on expression of the viral T antigens. Since polyomavirus large T antigen requires HSP70 binding and activation to promote cell cycle progression, we investigated whether MCC cells are sensitive to the HSP70 specific inhibitor MAL3-101 *in vitro*. Growth inhibition byMAL3-101 was measured for five MCV positive and four MCV negative as well as two non MCC cell lines by applying the MTS assay. We found that the cell lines displayed highly divergent sensitivities towards MAL3-101. In contrast to our initial hypothesis, however, MAL3-101 sensitivity did not correlate with the MCV status; this notion may reflect the recent identification of highly homologous polyomaviruses. By Annexin-V and DNA staining the effect of MAL3-101 was ascribed to the induction of apoptosis. Analysis of the expression profiles of all 14 HSP70 isoforms revealed a strong correlation of MAL3-101 sensitivity with expression of HSPA8 (hsc70), the most prominent isoform in all cell lines. To further establish MAL3-101 as a possible treatment for HSPA8 expressing Merkel cell carcinoma, we tested the drug in a MCC xenotransplantation model. Mice treated with MAL3-101 showed no side effects but -importantly – reduced tumor growth was evident. Our data warrant further studies to test whether MAL3-101 might be a therapeutic option for HSPA8 expressing Merkel cell carcinomas.

P203

Role of antiapoptotic Bcl-2 family members in the survival of melanoma cells and non-malignant human skin cells

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Melanoma is an aggressive skin tumor that is highly resistant to therapy in advanced stages. Targeted intervention in apoptotic regulation of melanoma cells may be an attractive strategy to overcome this obstacle. Here, we investigated the relevance of all antiapoptotic Bcl-2 family members for survival of melanoma cells in comparison to primary cells.

In primary human fibroblasts, knockdown of single antiapoptotic Bcl-2 proteins did not affect survival, as exemplified by RNAi-mediated inhibition of Bcl-2, Bcl-w, Bcl-xL, Mcl-1, and A1. However in melanoma cells, inhibition of Mcl-1 or A1 substantially induced apoptosis which was not the case when other antiapoptotic Bcl-2 proteins were knocked down.

Further analysis was carried out by systematic knockdown of two antiapoptotic Bcl-2 proteins simultaneously. In fibroblasts, apoptosis was induced by co-inhibition of Mcl-1 and Bcl-xL and of Bcl-xL and Bcl-w, however, the latter via induction of Noxa. In contrast, in melanoma cells, several different co-inhibitions led to cell death. Strikingly, inhibition of A1 together with Mcl-1 strongly induced apoptosis in all tested melanoma cell lines (up to 80% dead cells), whereas fibroblasts remained viable.

These results suggest that mitochondrial apoptosis in non-malignant cells is only activated when at least two antiapoptotic Bcl-2 proteins are inactivated, such asMcl-1 and Bcl-xL. In contrast, survival of melanoma cells depends on single antiapoptotic proteins, indicating that certain protective mechanisms to circumvent apoptosis may have been lost. Targeting A1 and Mcl-1 in melanoma cells could be a new therapeutic strategy to induce apoptosis in a tumor-specific manner.

P204 (V06)

Real-time imaging of cell cycle progression in melanoma

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While it is well appreciated that unrestricted proliferation is a cancer hallmark, the geometry and dynamics of cell cycle progression of individual tumor cells are not known. In the present study, we have made use of the fluorescence ubiquitination cell cycle indicator (FUCCI) system to assess the cell cycle dynamics in melanoma in real-time *in vitro* and *in vivo*. In the FUCCI system, cells transition from red (G1) to yellow (S) and green (S/G2/M), followed by a short period of fluorescence negativity during cytokinesis.

We first characterized the cell cycle behavior of melanoma cells during proliferation, migration, and in response to targeted therapy *in vitro*. This allowed us to measure the precise duration of G1, S and S/G2/M phases and to correlate cell cycle phases with migration. We then made use of our 3D-spheroid model composed of FUCCI-tagged melanoma cells. Initially, the ratio of red:green melanoma cells was roughly equal, and their distribution was random. Strikingly, within hours cycling green cells sequestered in a ring-like pattern at the periphery of the spheroid, while cells in the center remained in G1 (red). Treatment with the MEK1/2 inhibitor U0126, which causes G1 arrest in melanoma both in 2D and 3D-culture, induced homogeneous red fluorescence (G1) over the course of 24 h. To investigate the micro-anatomical distribution of cycling melanoma cells *in vivo*, FUCCI-tagged melanoma cells were xenografted into NOD/SCID mice. Tumors were allowed to grow until they were palpable and were then analyzed by confocal microscopy. Unexpectedly, we found clusters of differentially cycling subpopulations within the xenografts that were located next to each other, often with a sharp demarcation between the clusters.

Together, our data suggest that melanomas are composed of differentially cycling tumor cells in a subcompartment-specific distribution, which has implications for the evaluation of targeted therapies.

P205

Decreased cell viability and induced apoptosis in CTCL cells lines by non-steroidal anti-inflammatory drugs (NSAIDs)

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Cutaneous T cell lymphomas (CTCL) form a heterogeneous group of non-Hodgkin lymphomas with primary involvement of the skin. Even though early stages of CTCL are often indolent over long periods of time, advanced stages are refractory and difficult to treat. Death ligands (CD95L and TRAIL) critically contribute to lymphocyte homeostasis due to induction of apoptosis and may further represent safeguard mechanisms to prevent lymphoma development. In previous studies, we characterized CTCL cell lines as resistant to TRAIL-mediated apoptosis which was correlated to high c-FLIP expression. In the present study, we investigated the effects of non-steroidal anti-inflammatory drugs (NSAIDs) as acetylsalicylic acid, sodium salicylate and diclofenac in CTCL cell lines (HH and MyLa) as well as in tumor T cells from SzS patients. NSAIDs decreased cell viability and induced apoptosis, associated by caspase-3 processing. Decreased mitochondrial membrane potential and cytochrome c release were indicative for an involvement of intrinsic pathways. Downregulation of c-FLIP and caspase-8 processing clearly indicated an activation of extrinsic pathways. Finally, NSAIDs sensitized CTCL cells for

TRAIL-induced apoptosis. In conclusion, the study provides a rational for the use of NSAIDs as a potentially new therapeutic option for cutaneous T cell lymphomas.

P206 (V31)
Melanoma cells control synthesis of hyaluronic acid in peritumoral fibroblasts via TGFbeta, PDGF-AA and PDGF-CC: impact on melanoma cell proliferation

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Hyaluronan (HA), an ECM component, plays a pivotal role in tumor progression. Stroma-derived HA may support tumor cell proliferation and migration. Previously we have shown that soluble mediators from tumor cells of Malignant Melanoma (MM) elevate the synthesis of HA in stromal fibroblasts through the induction of HA-Synthetases (HAS) 1 and 2. Based on these findings we aimed to identify the functional impact for melanoma cells and the signaling pathways that are involved in this paracrine tumor-stroma interaction.

Using co-culture experiments we could demonstrate that MM cell lines Bro and HT144 show increased cell proliferation when grown on a layer of HA-secreting fibroblasts. Blocking of HA-synthesis in fibroblasts with 4-Methyl-umbelliferone abrogated this effect. Melanoma cell conditioned medium (MMCM) potently induceHAS1 and HAS2 mRNA and HA-synthesis in fibroblasts indicating that soluble factors released by melanoma cells are responsible for this effect. To identify the MM-derived mediators and their respective receptors on fibroblasts stimulations with recombinant candidate factors, siRNA transfections and function-blocking antibodies were used. The involvement of MM-derived metabolites (lactate) and several cytokines like IL-1β or bFGF could be excluded.

We could show that TGF-β1 is the stimulating mediator for HAS1 and silencing of TGFβ1 in Bro cells abrogated this induction. The growth factors PDGF-AA and PDGF-CC induce HAS2 expression in fibroblasts. Furthermore, silencing PDGF-A and PDGF-C mRNA in melanoma cells and/or blocking PDGFR-α on fibroblasts could reduce the observed stimulation by MMCM.

Taken together, melanoma-derived mediators TGFβ1, PDGF-AA and PDGF-CC stimulate HA-synthesis in stromal fibroblasts thus enhancing melanoma cell proliferation.

P207
Cell adhesion dependent regulation of the transcription factor c-Jun in melanoma

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The transcription factor c-Jun is a key player in the process of cell proliferation, apoptosis and differentiation in tumor progression. It forms dimers with other members of the transcription factor superfamily AP-1, influencing the expression of a multitude of genes involved in tumor development and metastasis, like MMPs, cyclinD1 and RACK1.

We revealed that loss of E-cadherin during development of melanoma leads to induction of c-Jun protein expression. Interestingly, the mRNA level of c-Jun was not affected, supposing that c-Jun is regulated on posttranscriptional level in melanoma. Here, we present data that the dynamic cytoskeletal network linked to E-cadherin is involved in the regulation of c-Jun protein. Immunofluorescence experiments with cytoskeletal disrupting agents taxol and nocodazole give a hint for cytoskeletal dependent regulation of c-Jun stability.

In a cascade where loss of E-cadherin leads to activation of the transcriptional regulator ETS-1 and consequently to RhoC expression, c-Jun is stabilized. The link between RhoC and c-Jun is indirect via the cytoskeleton. We conclude that loss of E-cadherin mediated cell-adhesions induce c-Jun protein expression by a multistep process, offering several possibilities of therapeutic intervention.

P208 (V33)
The role of protein S100 A9 and myeloid-derived suppressor cells (MDSC) in tumor metastasis

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Introduction: MDSC are subtypes of monocytes which can be found in tumor-bearing individuals. Due to their immuno-regulatory functions (inhibition of CD8+ cytotoxic T cells) they are counted to the immune-escape mechanisms. They belong to the group of CD11b+, Gr1+ und CD124+ leukocytes whereby a specific marker does not exist. We have already shown the presence of MDSC in a melanoma mouse model (B16 melanoma). The development and function of MDSC depends on the expression of the pro-inflammatory protein S100 A9.

Question: Does protein S100 A9 have impact on growth of primary tumors and/or metastasis?

Results: In tumor-bearing S100 A9 deficient mice (S100 A9-/-) the number of MDSC in blood was significantly decreased and T cell functions were not affected in comparison to wild type mice (WT). After injection of B16 melanoma, S100 A9-/- mice showed a significant lower primary tumor growth. Furthermore, PET-CT examination revealed less grade of metastasis in S100 A9-/- mice in comparison to WT (less lesions, less signal intensity).

Conclusion: **1** Protein S100 A9 is essential for the development of MDSC which regulate immune responses.

2 The absence of MDSC correlates with significantly reduced tumor-size and with lower FDG activity in PET-measurements.

3 Medicamentous inhibition of protein S100 A9 might open new therapeutical approaches in tumor treatment.

P209 (V05)
TLR-mediated inflammation promotes metastasis of B16-melanoma

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Toll-like receptor (TLR) stimulation was primarily developed as anti-tumor therapy. More recent data implicated TLR stimulation in the transition of pre-malignant lesions into cancer and outgrowth of metastases. Thus, TLR signaling may either promote or inhibit tumor growth and metastasis. Yet, the role of TLR on seeding of metastatic tumor cells is still unclear. Subcutaneous (s.c.) injection of 1.5 106 B16 melanoma cells significantly induced TLR2, -4, -7 and -9 expression, with different expression patterns on day 5, 12 and 14. On day 5 TLR2 was dominant, while TLR7 dominated on day 14. To determine whether this signaling was of biological relevance we transplanted the tumor into MyD88.KO-mice, deficient in TLR-signaling. MyD88.KO-mice had a 60% reduced tumor growth, and 4/18 tumors did not grow. As a second model we analyzed seeding of B16 melanoma cells after intravenous (i.v.) injection and found after 24 and 48 h a significant up-regulation of TLR4 and TLR7 in the lung tissue. As negative control we injected equal numbers of mononuclear cells. We used this model to identify the compartment responsible for the increased TLR expression. We separated tumor cells from lung tissue cells via FACS, 24 and 48 h after intravenous injection of DiD-labeled B16 melanoma cells. The data revealed host derived cells as the main source of the increased TLR7 expression in metastatic lungs. Thus, by initiating TLR-signaling tumor cells create amilieu that promotes their own seeding.

P210
Sensitization of melanoma cells for TRAIL-induced apoptosis by indirubin 8-Rha-beta correlates with enhancement of extrinsic and intrinsic pathways

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Background: No effective therapy is available for metastatic melanoma so far. An anti-tumour activity of indirubin is known from traditional Chinese medicine, and its derivative 8-Rha-beta has been described as a cyclin-dependent kinase inhibitor. However, the molecular basis underlying 8-Rha-beta-induced apoptosis remained elusive. TNF-related apoptosis-inducing ligand (TRAIL) is known to trigger apoptosis in a variety of human cancer cells, while normal cells are largely spared. However, prevalent or inducible resistance prevented its efficient use in cancer therapy so far. TRAIL resistance in melanoma cell lines is frequently associated with downregulation of its agonistic receptors DR4 and DR5.

Methods: TRAIL-sensitive melanoma cell lines A-375 and Mel-HO were compared to permanently resistant MeWo and Mel-2a as well as to cell lines selected for death ligand resistance A-375-TS, Mel-HO-TS (TRAIL-selected) and A-375-CS, Mel-HO-CS (selected with an agonistic CD95 antibody, CH-11, for resistance to the death ligand CD95L).

Results: Both death ligand-sensitive cell lines (A-375 and Mel-HO) responded with enhanced apoptosis to combinations of death ligands (TRAIL, CH-11) with8-Rha-beta. The indirubin was further able to sensitize resistant Mel-2a andA-375-TS (DR4+, DR5+) for death ligand-induced apoptosis. In contrast, MeWo and Mel-HO-TS (DR4-, DR5+) remained without effect. The unraveling of proapoptotic signaling pathways in A-375-TS revealed strong enhancement of the effectorcaspase-3 in the combination. Significant loss of the mitochondrial membrane potential, release of cytochrome c and apoptosis-inducing factor (AIF) as well as processing of caspase-9 was evident for activation of intrinsic apoptosis pathways. On the other hand, enhanced surface expression of DR4 and DR5 as well as processing of initiator caspase-8 was indicative for activation of extrinsic apoptosis pathways. Remarkably, this combination was able to overcome an apoptosis block due to ectopic Bcl-2 overexpression. The effects may be explained by downregulation of antiapoptotic proteins Mcl-1 and XIAP as well as by activation of the master regulator p53 seen in course of 8-Rha-beta treatment.

Conclusions: Apoptosis resistance to TRAIL may be overcome by kinase inhibitors, and the indirubin 8-Rha-beta appears as a promising therapeutic strategy for melanoma cells, dependent on their expression of TRAIL receptors.

P211 (V21)
A seven-marker signature predicts the clinical outcome of malignant melanoma and offers new approaches to targeted therapy

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Background: Cutaneous malignant melanoma (MM) represents the leading cause of skin cancer death in industrialized countries. Clinical and histological variables such as tumor thickness, ulceration and invasion of the sentinel node are known to be prognostic parameters in patients with MM. However, the potential relevance of other biological variables concerning the metastatic risk of MM is still poorly understood.

Methods: Using tissue microarrays (TMAs), we retrospectively analyzed samples from 465 patients; 364 with primary MM, 39 with metastases, and 62 with benign nevi. Clinical follow-up data (AJCC 2002 staging, overall and recurrence-free survival and tumor therapy) were available for all patients with primary MM. We investigated a panel of 70 immunohistochemical (IHC) antibodies for cell cycle, apoptosis, DNA mismatch repair, differentiation, proliferation, cell adhesion, signaling and metabolism. A marker selection procedure based on univariate Cox regression and multiple testing correction was employed to correlate the IHC expression data with the clinical follow-up. The model was thoroughly evaluated with two different cross validation experiments, a permutation test and multivariate Cox regression analysis. Additionally, the predictive power of the signature was tested on an external validation set, consisting of a second TMA of patients from a different hospital.

Results: A signature of seven biomarkers was found to be an independent predictor for overall and recurrence-free survival in patients with MM. In particular, three of these markers were shown to offer direct therapeutic implications. Remarkably, the seven-marker signature could also predict those patients with worse prognosis despite small tumor thickness.

Conclusions: Our seven-marker signature is closely associated with the prognosis of patients with MM and offers direct therapeutic implications. Prospective clinical studies are going to show if the identified signature may be an appropriate clinical tool to improve predictive evaluations and targeted therapy in patients with MM.

P212
Interferons affect cellular viability and class I major histocompatibility complex expression in both MCV positive and negative merkel cell carcinoma cell lines

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Merkel cell carcinoma (MCC) is a rare, highly aggressive neuroendocrine skin cancer which primarily affects elderly and immune suppressed individuals. The presence of merkel cell polyomavirus (MCV) in about 80% of MCC suggests an involvement of the virus in the pathogenesis of MCC; a notion which we could recently substantiate by demonstrating oncogene addiction of MCC to the MCV T-antigens (TA). Interferons (IFN) are frequently used for immunotherapy of cancer and viral diseases. Moreover, inhibition of polyomavirus large TA expression by IFN has been demonstrated for JC and BK polyomaviruses.

Therefore, we investigated the effects of Multiferon™ (MFN; different IFN alpha subtypes), IFN β and IFN γ on four MCV positive and four negative MCC cell lines. In this regard, MFN and IFN β possessed anti-proliferative effects on six of these eight cell lines detected by a MTS based cell proliferation assay and cell cycle analysis. ForIFN γ, only four of the eight cell lines were sensitive to the cytokine. In general, the anti-proliferative effects of MFN and IFN β were stronger than the effect of IFN γ. Although it was recently shown that TA expression is necessary for the maintenance of MCV-positive MCC, a direct link between IFN related inhibition of viral TA and cell viability could only partially be demonstrated. Thus, other mechanisms have also to be involved. Moreover, in addition to directly affecting MCC cell proliferation, IFNs strongly reinduce MHC class I expression in MCC cells. Flow

cytometry demonstrated a reinduction of MHC class I expression upon IFN treatment in three MHC class IMCV positive cell lines and an increase in MHC class I expression in cell lines that were characterized by a weak expression prior to treatment. Importantly, the increase or induction of MHC class I expression could also be demonstrated *in vivo* in xenotransplantation models. These results imply that IFN treatment has both a direct and an indirect effect in MCC and should be applicable in a general manner, i.e. irrespective of the MCV status of the patient.

P213
Role of micro RNA 372 in melanoma progression

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Dysregulation of micro RNAs (miRNAs) has been reported in various types of cancer including malignant melanoma. Recently, we accomplished a miRNA expression screening of primary melanomas ($n = 10$) and cutaneous melanoma metastases ($n = 20$) and identified a series of interesting candidate miRNAs, which might help to explain tumor aggressiveness in this tumor. Of 14 miRNAs, which were found to be differentially regulated, miR-372 showed the most significant downregulation in metastatic samples as compared to primary melanomas, suggestive for an inhibitory role of this miRNA in melanoma metastasis. Unexpectedly, a recent report showed that miR-372 enhances proliferation of tumor cells in other types of cancer. In line with this, transient transfection of SK-Mel-147 melanoma cells with mature miR-372 in our experiments enhanced cellular proliferation. In search for another target molecule of miR-372 which might explain its role in metastatic lesions, we identified vascular endothelial growth factor (VEGF), a major inducer of tumor angiogenesis and inhibitor of tumor cell apoptosis. VEGF has been described to be upregulated during melanoma progression and metastasis. Thus, downregulation of miR-372 might be a major prerequisite for enhanced VEGF expression during melanoma progression. In further experiments, we showed that miR-372 transfection of melanoma cells significantly reduced protein levels of VEGF, indicating that VEGF is indeed a target for miR-372 in melanoma cells. These findings are suggestive for a functional role of miR-372 in melanoma angiogenesis. Taken together, our study provided evidence that VEGF is regulated by miR-372 and downregulation of miR-372 in metastatic lesions might promote VEGF expression during tumor progression. These data are encouraging for future studies focussing on the regulation of this miRNA.

P214
Melanoma-derived VEGF triggers an acute endothelial cell activation

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Tumor cell extravasation is a critical step in the metastatic cascade, which requires the interaction between the tumor cell and the endothelium. In previous studies we demonstrated that tumor-derived MMP-1 or tumor-mediated thrombin generation via tissue factor are potent secretagogues to stimulate endothelial cells (EC). In this study, we show that supernatants from high-invasive melanoma cells induce an acute EC activation measured by von Willebrand factor (VWF) release via Weibel-Palade body exocytosis, whereas supernatants from low-invasive cells fail to activate ECs. Upon this EC activation, the regular repressive function on inflammation and coagulation subsides and the endothelium converts to a proinflammatory and procoagulatory surface. Analysis of proteins secreted into the supernatants of both melanoma cell types identified differential expression of vascular endothelial growth factor (VEGF). VEGF was expressed by high- but not by low-invasive melanoma cells, and VEGF secretion strongly correlated with the induction of EC activation. Inhibition of VEGF by a humanized monoclonal anti-VEGF antibody or knock-down by siRNA in melanoma cells led to rigorous blocking of EC activation. Interestingly, the EC activation was also blocked when stimulating EC with supernatants of MMP-1 or MMP-2 knock-down melanoma cells. The analysis of VEGF in MMP-1 or MMP-2 knocked-down melanoma cells showed a reduced VEGF expression in these cells, correlating with reduced EC activation. Taken together, these results indicate an important role of VEGF in acute EC activation and provide clear evidence that MMPs are involved in the regulation of the VEGF expression in melanoma cells.

P215
Autoantibodies against CD28 – a new prognostic marker in malignant melanoma with impact on therapies with interferons?

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Background: Activation of T cells occurs through the engagement of both the T cell receptor (TCR) and CD28 on the T cell by the major histocompatibility complex peptide and B7 family members on the antigen-presenting cells (APC) respectively. Both are required for production of an effective immune response; in the absence of CD28 co-stimulation, T-cell receptor signaling alone results in anergy. 'Superagonists' can activate a T cell directly by binding CD28 without the need of further signals. Antibodies (abs) against CD28, which could possibly act as superagonists, occur rarely in a diversity of autoimmune diseases such as multiple sclerosis or rheumatism, in infections and in patients with atopic diseases. The occurrence and impact of CD28-abs in malignant melanoma and other neoplasms has not yet been investigated.

Methods: Full-length human CD28 was amplified by PCR from Jurkat cell line and cloned in expression vector pSfi-FLAG. Expression was done as stable cell line inHEK293. CD28-FLAG was obtained by lysis of the cells with 10 mM Tris pH 8. NuncMaxisorb ELISA plates were coated with anti-FLAG (1:2500, 4°C, 16 h) followed by blocking with 1.5% gelatine in wash buffer (1 h RT) and incubation with the recombinant CD28-FLAG protein (10 μg/ml; 50 l; 1 h RT). Between each step of the procedure intensive washing with TBS/0.1% Tx100 occurs. Human serum was added at 1:100 for 1 h RT, followed by anti-human-IgG-Biotin (1:2500 1 h RT) and Strep-POX (1:50000 1 h RT). Development with OPD was done for 10 min RT, stopped with HCl and measured by 490 nm. Values with an OD >10× background were considered as positive.

Serum samples from 101 patients with malignant melanoma, 152 patients with hay fever or asthma bronchiale, 78 patients with psoriasis vulgaris, 46 patients with plasmocytoma and 48 healthy blood donors were investigated.

Results: Sixteen of 101 (15.84%) patients with melanoma showed CD28-abs, whereas their prevalence in other groups was considerably lower (atopic diathesis 6.57%; psoriasis 3.84%; blood donors 2.08%, plasmocytoma 8.69%). CD28-antibodies in melanoma patients were mainly found in higher stages of the disease. Remarkably, patients under immunotherapy with interferon in an adjuvant setting showed CD28abs in an even higher percentage (35.71%). In some patients a sero-conversion parallel to tumor progression could be observed.

Discussion: Further observation of the clinical course is needed to investigate whether the presence of CD28abs is associated with a shortened progress-free and overall survival or not. Interferons seem to induce the production of CD28abs. It cannot be rouled out that CD28abs stimulate regulatory T-cells and lead to suppression of T-cell answers resulting in immunosuppression in melanoma patients. It is thinkable, that this effect predominantly occurs in patients receiving interferons. *In vitro* T-cell models may further elucidate the role of CD28abs in melanoma.

P216
Differential expression of ZO-1 in melanoma and nevi and their tumor microenvironment – an indicator of malignancy

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ZO-1 is a multifunctional protein that is involved in the formation of Tight Junctions as well as other cell-cell-junctions, cell signalling, regulation of cell growth and cellular differentiation. In a previous report a contribution of ZO-1 to melanoma progression was proposed, i.e. the knock-down of ZO-1 resulted in decreased invasiveness of melanoma spheroids. To further elucidate its role in melanoma we investigated ZO-1 in tissue sections of MM, dysplastic nevus cell nevi (NZN) and Spitz nevi (SN) as well as several melanoma cell lines.

Surprisingly, we observed a presence of ZO-1 in all MM as well as the majority of NZN, SN and melanoma cell lines by using immunofluorescence microscopy, Western blotting and RNA analysis. This indicates that different ZO-1 expression levels rather than simply its presence may mark invasiveness. In fact, we found a correlation of increased ZO-1 immunoreactivity in invasive areas of MM and Breslow Index as a marker for tumor progression. Invasion assays using melanoma cell lines expressing different levels of ZO-1 further supported this hypothesis.

Of note, we observed pronounced alterations of ZO-1 expression in the epidermal tumor microenvironment (TME) of MM compared to NZN. While 90% of MM showed a remarkable up-regulation of ZO-1, i.e. expression in all epidermal layers, this was seen in none of the NZN, even dysplastic ones. SN showed heterogeneous results. The TME has gained significant interest due to the substantial influence of the interaction of tumor cells with their environment for tumor progression. Looking for putative consequences of presence and absence of ZO-1 in the TME we performed knockdown studies in keratinocytes. They revealed an influence of the presence of ZO-1 on cytokine levels released by the cells which is likely to result in an alteration of the inflammatory milieu surrounding the tumor. In addition, ZO-1 expression is associated with barrier function of keratinocytes.

In conclusion, we suggest a correlation of ZO-1 protein expression levels in melanoma cells with their invasiveness. In addition, we show that the presence of ZO-1 expression in lower layers of the epidermal TME is related to malignancy of the tumor. Therefore ZO-1 levels in the tumor and ZO-1 expression in TME are indicators for malignancy. The latter could be used for diagnostic delineation between melanomas and nevi.

P217
Modulation of NOXA and MCL-1 as a tactic for sensitizing melanoma cells to the BH3-mimetic ABT-737

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Melanoma's drug resistance is commonly attributed to ineffective apoptosis pathways. Inhibiting anti-apoptotic BCL-2 and its relatives is an attractive strategy for sensitizing lymphoid malignancies to drugs but it has been largely unsuccessful for melanoma and other solid tumors. ABT-737 (Abbott Laboratories, Ludwigshafen, Germany), a small-molecule BH3-mimetic, selectively inhibits BCL-2, BCL-XL and BCL-w and shows promise for treating leukemia, lymphoma and small cell lung cancer. Melanoma cells are insensitive to ABT-737 but MCL-1 inhibition reportedly increases the sensitivity of other tumors to the compound, so we investigated its efficacy in the context of melanoma. Indeed, direct inhibition of MCL-1 by shRNA-mediated knockdown or indirect inhibition by NOXA overexpression, strongly sensitized melanoma cells toABT-737. NOXA-inducing cytotoxic drugs also strongly sensitized melanomas to ABT-737 (although not vice versa) so this is a therapeutic strategy worth exploring. Surprisingly, the strong sensitization to ABT-737 obtained *in vitro* by NOXA overexpression or MCL-1 knockdown was not mirrored in xenografts, even though there were systemic effects on platelets and leukocytes, and the tumors were vascularized. Explants from unresponsive xenografts remained sensitive *in vitro*, suggesting insufficient exposure to the drug *in vivo*. Three-dimensional spheroids grown *in vitro* from melanoma cells were also sensitized to ABT-737 by overexpression of NOXA. However, resistance developed through loss of NOXA expression and larger 3D-spheroids were less amenable to treatment, suggesting that diffusion of ABT-737 into the tumor is limiting. The latter issue may be obviated through use of more bioavailable relatives of ABT-737.

P218
Utilization of the auto fluorescent drug Rose Bengal as a novel approach for the investigation of drug effects within solid tumors in 3D culture and *in vivo*

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Rose Bengal (RB; 4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluoresceindisodium) hasbeen reported to promote cell death *in vitro* and in patients. Except for its phototoxicity there were no side effects. However, the mechanism of action is not yet understood.

Here, we show that RB indeed has a cytotoxic effect on melanoma cells but not fibroblasts in the absence of light or upon exposure to red light (633 nm), whereas exposure to UV- or green light (561 nm) caused profound phototoxicity within minutes. RB has an IC50 of 32–64 μM and causes cell death without prior disruption of the cell cycle. Annexin V/DAPI-staining of melanoma cells upon RB-treatment showed that this is rather due to necrosis than apoptosis. Furthermore, RB inhibits migration dose-dependently. We utilized our previously described 3D spheroid model, which mimics melanoma architecture and microenvironment, and RB'sautofluorescence (575–695 nm) for simultaneous imaging of the drug and GFP-labelled melanoma cells. Here we show that RB causes dose- and time-dependent cell death of both proliferating and invading cells of the spheroid. We injected RB intralesionally into GFP-expressing human melanoma xenografts on SCID mice. Using multi-photon microscopy, we were able to track RB-affected and unaffected melanoma cells *in vivo*. Similar to our culture experiments, melanoma cells once affected by RB (fluoresceing red) lose their green fluorescence (GFP) and shrink in comparison to nearby GFP-positive likely unaffected melanoma cells. This indicates that RB is also cytotoxic *in vivo*, but its penetration through the tumor maybe hampered. Ongoing experiments will further elucidate this phenomenon.

Taken these results together, we showed that Rose Bengal causes necrosis rather than apoptosis in melanoma in 2D and 3D cell culture as well as *in vivo*. Importantly, taking advantage of the autofluorescence of RB and using multi-photon microscopy, we developed a novel model for the investigation of drug effects within solid tumors.

P219

Regulation of proliferative activity and proinflammatory chemokine expression in Hgf-Cdk4R24C mouse melanoma cell lines

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Human melanoma cell lines frequently produce the proinflammatory chemokine CXCL8/IL-8 which acts as an autocrine growth factor and promotes the recruitment of myeloid immune cells into the tumor microenvironment. Here we investigated the role of CXL2/MIP2, the mouse homologue of human CXCL8, in melanoma cell lines established from primary tumors in Hgf-Cdk4R24C mice. We found that different melanoma cell lines produce varying amounts of CXCL2 constitutively and following TNFα stimulation. High CXCL2 production was associated with strong proliferation *in vitro* and aggressive growth in immunocompetent syngeneic C57BL/6 mice *in vivo*. Using small molecule inhibitors we are currently investigating the impact of different oncogenic signaling pathways on the constitutive and inducible secretion of CXCL2 and the proliferative activity in these cell lines. Our investigations in this experimental model contribute to an understanding how melanoma cells acquire a proinflammatory phenotype and how this supports aggressive growth and metastatic behaviour in immunocompetent syngeneic hosts *in vivo*.

P220

Discrepancy between cKIT mutations *in vivo* and *in vitro* in acro lentiginous and mucosal melanoma

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Introduction: KIT is a receptor tyrosine kinase important for growth and survival functions. cKIT mutations are present in 10–20% in acro lentiginous (ALM) and mucosal melanomas (MM). cKIT is a target of the kinase inhibitor Imatinib. This drug was successfully used in KIT-mutant melanoma patients.

Objectives: *In vitro* investigations of melanoma cells shall help explain the *in vivo* responses towards cKIT targeted therapy. However, cKIT mutated melanoma cells are difficult to grow in culture. To date, extensive data are still lacking.

Material and Methods: Biopsies of 31 ALM and MM patients were available. Two of these patients were treated with Imatinib. Cells from these tissues were taken into culture. DNA was extracted from all cultured cells and the corresponding paraffin embedded tissues. cKIT exons 9,11,13,17,18 were tested for mutations. Additionally, one of the 31 cells was clonally expanded in an early passage. Subsequently, the cKIT mutation status was analysed for 60 of its subclones.

Results: Wild-type cKIT was detected in 30 cultured cells and 26 paraffin derived DNA. For three MM and two ALM, activating cKIT mutations were found in exons 11 and13. Two of these patients were responsive to Imatinib. In one culture mutated cells were found in the early passage. This mutation was lost after further passaging. It was also not maintained in any of the tested 60 subclones derived from single cells.

Conclusion: We confirm that KIT mutant melanoma patients can respond successfully to Imatinib. However, the mutation is lost *in vitro*. This discrepancy implies a heterogeneity for cKIT mutations within melanoma lesions. *In vitro* culturing probably triggers dominance for wild-type over mutated cells. This heterogeneity might explain the limited response duration of cKIT targeted therapy.

P221 (V15)

Inhibition of melanoma cell growth *in vitro* and *in vivo* by a selective oncolyticadenoviral vector with doxycycline-inducible expression of TRAIL

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The high mortality of melanoma demands the development of new strategies for targeting metastatic melanoma cells, and gene therapy may be considered provided improvements in efficacy and selectivity. Apoptosis deficiency has been reported as acritical factor of melanoma therapy resistance. Overcoming apoptosis deficiency of melanoma cells appears as particularly promising. TNF-related apoptosis inducing ligand (TRAIL) has been shown by us as highly effective for apoptosis induction in melanoma cells and may apply for gene therapy due to its selective impact on tumor cells.

We have constructed two conditional replication-competent adenoviral vectors for inducible expression of TRAIL (AdV-TRAIL) and for CD95L/FasL (AdV-CD95L). A variant viral E1A protein and the deletion of E1B aimed at the general restriction of viral replication to tumor cells. In particular, the replication gene E1A is controlled by a tyrosinase promoter with high selectivity for melanoma cells. The tetracycline/doxycycline-responsive transactivator rtTA and the respective death ligand gene are controlled by a bidirectional tetracycline-inducible promoter. Apoptosis induction 24 h after adenoviral transduction was monitored by a Cell Death Detection ELISA. Lysis of tumor cells in course of viral replication was investigated by a cell killing assay using crystal violet staining of adherent cells 5 days after adenoviral transduction as well as by real time cell analysis (RTCA). Growth of tumors established in nude mice was monitored after intratumoral injections of AdV-TRAIL.

AdV-TRAIL mediated strong expression of E1A and doxycycline-dependent induction of TRAIL selectively in melanoma cells. In AdV-TRAIL transduced melanoma cells TRAIL was abundantly expressed after induction with doxycycline and was detectable on the cell surface. Transduction of melanoma and non melanoma cells with AdV-TRAIL resulted in selective lysis of melanoma cells and doxycycline-dependent induction of apoptosis. In contrast, non-melanoma cells and normal human melanocytes appeared as protected. Comparison of AdV-TRAIL withAdV-CD95L revealed largely similar efficacies of both death ligands for melanoma cells *in vitro*. In melanoma xenotransplantation models, AdV-TRAIL demonstrated its efficacy by significant growth reduction of established melanomas after intratumoral application. Significant reduction of melanoma growth in nude mice was also observed in xenotransplants established from partially AdV-TRAIL transduced Mel-2a cells indicating viral replication and production of infective viral particles *in vivo*. Melanoma cell killing by AdV-TRAIL could be further improved *in vitro* by combinations with chemotherapeutics.

Conclusions: We demonstrate that melanoma cells can be efficiently targeted by death ligand-based gene therapies, and possible resistance may be overcome by combined chemotherapy.

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S100A7 (psoriasin) and S100A15 (koebnerisin) marks tumor progression during skin carcinogenesis

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The human S100A7 (psoriasin)/S100A15 (koebnerisin) subfamily is regulated with epidermal maturation and epithelial tumor progression. We implemented a corresponding mouse model to understand S100A7/S100A15 regulation and function. By customizing specific antibodies, expression of mouse S100A7A15 in both skin and isolated keratinocytes is confined to both basal and differentiating epidermal cells and reflects the expression pattern of both corresponding human proteins during normal skin maturation. To validate the mouse-human analogy for epithelial carcinogenesis, we analyzed mS100A7A15 in skin tumors derived from the two-stage carcinogenesis model. mS100A7A15 expression is induced in benign tumors, decreases with tumor progression and shows a focal expression in malignant squamous cell carcinomas. mS100A7A15 is upregulated in RAS-active tumor cell lines and oncogenic ras/EGFR induce mS100A7A15 in keratinocytes. Further, mS100A7A15 expression is decreased in p53-null tumor cell lines and is regulated dependent on p53 in keratinocytes. We conclude that the corresponding human S100A15/S100A7 subfamily is co-regulated during epidermal carcinogenesis suggesting a joint function in tumor progression. Thus, mS100A7A15 mirrors the human S100A7/S100A15 subfamily during normal skin maturation and epidermal tumorigenesis and provides a valid model to study its normal biological function and role in tumor progression.

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Integrin-mediated interactions between platelets and B16 melanoma cells augment tumor cell adhesion and promote lung metastasis formation

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Fundamental aspects determining the molecular basis for reciprocal interactions between metastasizing tumor cells and soluble components of the coagulation cascade have recently become understood. However, the involvement of platelets, the cellular component of thrombus formation, in the pathogenesis of cancer metastasis is still poorly comprehended.

We demonstrate that integrin-mediated interactions between platelets and tumor cells are an important contributing factor in the early phase of B16 melanoma (B16M) lung metastasis *in vivo*. As determined by *in vivo* and *in vitro* bioluminescence analysis the depletion of platelets after i.v. inoculation of luciferase-transduced murine B16M (B16M-luc) into syngeneic C57BL/6 mice resulted in a more than 30% decrease in micrometastasis to the lung.

While we observed no significant platelet mediated effect on the growth of subcutaneously implanted B16M tumors *in vivo* nor the proliferative and migratory capacities of B16M cells *in vitro*, we determined an up to 50 fold augmentation in the adhesion of melanoma cells on immobilized platelets under static condition. Importantly, we also observed a significant decrease in B16M adhesion to microvascular endothelium after platelet depletion *in vivo*, as determined by intravital microscroscopy analysis. Blocking mAb to alphaV integrin, an adhesion molecule highly expressed on 97% of B16M and blocking mAb to GPIIbIIIa, the most abundant platelet adhesion receptor, both significantly abrogated the platelet-mediated increase in *in vitro* B16M adhesion under both static and shear flow conditions while fibrinogen, the major GpIIIaIIb ligand, critically sustains B16M static adhesion *in vitro*.

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 molecules involved in this process may represent a promising strategy for therapeutic intervention.

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Identification of a chemokine expression signature associated with melanoma progression

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Development of distant metastases is the most important step in the progression of cutaneous melanoma; formation of metastases reduces outcome prognosis of affected patients significantly. Nevertheless, only little is known about mechanisms regulating tumor cell migration and the initial steps leading to the development of metastasis. Chemokines seem to play an important role in facilitating and guiding tumor cell motility as well as in targeting tumor cells to distinct metastatic sites. By using microarray technology, we have analyzed chemokine expression patterns in the stroma of primary tumors in a SCID mouse human melanoma xenotransplantation system. We were able to identify differentially expressed chemokines in the tumor stroma when compared to normal skin. In a next step, expression of corresponding human chemokines was analyzed by using real-time PCR on samples from human primary melanomas, stage T1 to T4.

We found chemokines whose expression levels correlated significantly with tumor thickness (and tumor stage). Interestingly, the identified chemokines have not yet been reported to be of prognostic importance in human melanoma. Therefore, these differentially expressed chemokines might serve as new prognostic markers for cutaneous melanoma.

P225

Oncogenic BRAFV600E promotes anchorage-independent survival of human melanocytes

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Normal cells are dependent upon integrin-mediated adhesion to the extracellular matrix for cell proliferation and survival. A trait of malignant cells is their ability to undergo anchorage-independent growth. Here, we demonstrate that the constitutively active B-RAFV600E present in a significant number of melanomas protects primary human melanocytes from anoikis, a form of apoptosis induced by lack of adhesion to an extracellular matrix. Moreover, expression of B-RAFV600E, but not wild type B-RAF induced dramatic morphological changes in primary human melanocytes. In particular, B-RAFV600E expressing melanocytes displayed fewer dendrites, appeared rounded and the majority detached from the culture flask. Importantly, the loss of adhesion induced by BRAFV600E did not induce anoikis, with 80–90% of suspension melanocytes remaining viable after transduction. Our findings demonstrate that constitutively active B-RAFV600E promotes anchorage-independent growth of primary human melanocytes. The impact B-RAFV600E on melanocyte adhesion and survival could conceivably increase cell motility and may account for the unique histopathological characteristics of B-RAFV600E melanomas, including upward migration of cells into the epidermal layer and intraepidermal 'nest' formation. Taken together, our data highlight that activated B-RAF regulates the adhesion and survival of human epidermal melanocytes.

P226

Interferon-γ induces growth arrest and senescence in cancer

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We previously demonstrated that interferon-γ (IFN-γ)-producing, tumor-specific Th1 cells prevent cancer in T antigen (Tag)-expressing RIP1-Tag2 mice. Th1 cell-based tumor prevention and therapy was

independent of CD8+ cytotoxic T cells or destruction of Tag-expressing cancer cells, and occurred in the absence of detectable apoptosis *in vivo*. Instead, Th1 cells reduced angiogenesis and arrested proliferation of tumor cells in RIP1-Tag2 animals by a mechanism that strictly required IFN-γ signalling. To elucidate the underlying principles, we investigated the direct influence of IFN-γ on cancer cell proliferation, apoptosis and senescence in an *ex vivo* approach. We isolated cancer cells from RIP1-Tag2 mice and characterized their identity using differentiation markers, i.e. synaptophysin, a primitive differentiation marker, insulin, an intermediate differentiation marker, and the glucose transporter Glut2, a late differentiation marker. Carcinoma cells were then treated with physiological doses of recombinant mouse IFN-γ for 72–96 h. Subsequently, we determined BrdU incorporation to quantify proliferation, TUNEL staining and caspase 3/7 activity as indicators of apoptosis, and senescence-associated β-galactosidase (SA-β-gal) and nuclear localization of heterochromatin protein 1 γ(HP1-γ) as marker of senescent cells. IFN-γ dose-dependently reduced the proliferation of cancer cells, at 100 ng/ml by 70%. The antiproliferative effect of IFN-γ was accompanied by induction of SA-β-gal activity in ≥20% of the cancer cells. Quantification of nuclear translocation of HP1-γ confirmed that IFN-γ treatment induced a senescent phenotype. In sharp contrast, IFN-γ did not induce any sign of apoptotic damage of the nuclei or caspase 3/7 activation *in vitro* (apoptotic cells remained ≤5%). Double-staining with SA-β-gal/synaptophysin revealed that the senescent phenotype occurred in synaptophysin+ cancer cells. Together, the data show that specific Th1 cells directed against a tumor-associated antigen arrest tumor development by IFN-γ-mediated inhibition of cell proliferation, in the absence of major cytotoxic effects. As IFN-γ prevented carcinoma development without any sign of apoptosis also *in vivo*, induction of cancer cell senescence may be a central mechanism underlying the anti-tumor effects of IFN-γ-producing Th1 cells.

P227
Tumor-specific T helper 1 (Th1) cells prevent phenotypical transformation of epithelial cells into cancer *in vivo*

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Immune-mediated control of cancer and tumor eradication critically depend on interferon-gamma (IFN-γ) and tumor necrosis factor (TNF). Using C3H mice developing islet cancer due to aberrant regulation of p53 and Rb, we analyzed the effect of IFN-γ- and TNF-producing Th1 cells on the differentiation and proliferation of the developing cancers. *In vivo* analysis of the proliferation marker Ki67 as well as direct *ex vivo* culture of isolated epithelial cells showed that Th1 cells suppressed proliferation of the potential cancer cells by 80% to 90%. As proliferation and terminal differentiation are inversely regulated, we next investigated the expression of primitive (synaptophysin, Syn+), intermediate (insulin, Ins+) and late (glucose transporter Glut2, Glut2+) differentiation markers in developing cancers of eithersham- or Th1 cell-treated mice. Within 12 weeks, cancers of sham-treated mice did not only grow 10-times faster, they also lost >90% of the late differentiation markerGlut2, and about 50% of the insulin. In contrast, all cancer cells remained positive for the primitive differentiation marker synaptophysin. These data directly show that the rapid proliferation correlated with a loss of cell differentiation. Epithelia of Th1cell-treated mice were growth arrested and they fully preserved their functional phenotype (Syn+, Ins+, Glut2+). As another hallmark of cancer is growth factor-independent and ectopic growth, we injected isolated tumor cell lines after three passages into immunosuppressed SCID mice. Cancer cells from Th1-treated mice remained growth-arrested and failed to expand *in vitro* or after transfer into SCID mice. In sharp contrast, cancer cells from sham-treated mice expanded rapidly *in vitro* and established rapidly growing cancers when transferred into SCID mice. Th1 immunity inhibits not only cancer cell proliferation but preserves the functional phenotype and differentiation of oncogen-driven epithelia. These data show for the first time, that adaptive immunity can prevent cancer by inhibiting proliferation without cancer cell destruction.

P228
The dual role of IFN-alpha in TRAIL-induced apoptosis of melanoma cells

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Human peripheral blood leukocytes acquire the cytotoxic molecule TNF-related apoptosis-inducing ligand (TRAIL) in response to IFN-alpha treatment. Since IFN-alpha is used adjuvantly in melanoma therapy, we asked the question whether the induction of TRAIL+ immune cells has a role in preventing disease progression. To this end we analyzed the susceptibility of established melanoma cell lines and such generated from metastases of stage IV melanoma patients to TRAIL. Over night treatment of melanoma cells with soluble TRAIL induced apoptosis ranging from 6% to 54%, as determined by Annexin V/PI staining. Since TRAIL acts via TRAIL receptors (TRAIL-R) we analyzed the TRAIL-R expression pattern on melanoma cells by flow cytometry. We generally detected moderate to high levels of the pro-apoptoticTRAIL-R2, but little to no expression of TRAIL-R1, -R3 and -R4. This was reflected in the TRAIL-R2 mRNA levels, which were generally 2-log higher compared to the other receptors. Interestingly, the magnitude of TRAIL-induced apoptosis did not correlate with TRAIL-R mRNA levels or protein surface expression. Since the role of IFN-alpha in melanoma may not be confined to leukocyte effector cells, we also studied its effects on melanoma cells. Treatment of melanoma cells with IFN-alpha alone inhibited proliferation but did not induce apoptosis. IFN-alpha pre-treatment, however, enhanced subsequent TRAIL-induced apoptosis, suggesting effects on TRAIL-R expression or other molecules of the apoptotic cascade. In summary our findings suggest that by inducing cytotoxic TRAIL on effector leukocytes and enhancing melanoma cell susceptibility to TRAIL, the role of IFN-alpha in melanoma therapy may be a dual one.

P229
Multiple oncogenic mutations and clonal relationship in spatially distinct benign human epidermal tumors

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Malignant tumors result from the accumulation of genetic alterations in oncogenes and tumor suppressor genes. Much less is known about the genetic changes in benign tumors. Seborrheic keratoses (SK) are very frequent benign human epidermal tumors without malignant potential. We performed a comprehensive mutational screen of genes in the FGFR3-RAS-MAPK and PI3K-AKT pathways from 175 SK, including multiple lesions from each patient. SK commonly harboured multiple bonafide oncogenic mutations in FGFR3, PIK3CA, KRAS, HRAS, EGFR, and AKT1 oncogenes but not in tumor suppressors genes TSC1 and PTEN. Despite the occurrence of oncogenic mutations and the evidence for downstream signalling, we did not find induction of senescence or a DNA damage response. Array CGH analysis revealed that SK are genetically stable. The pattern of oncogenic mutations and X-chromosome inactivation departs significantly from randomness and indicates that spatially independent lesions from a given patient share a clonal relationship. Our findings show that multiple oncogenic mutations in the major signaling pathways involved in cancer are not sufficient to drive malignant tumor progression and therefore suggest an important role for changes in genomic architecture. Furthermore, our data provide new clues on the origin and spread of oncogenic mutations in tissues, suggesting that apparently independent (multicentric) adult benign tumors may have a clonal origin.

P230
Clues to organ-specific metastasis: liver endothelial-specific differentiation and trans-differentiation mediated by malignant melanoma and hepatocellular carcinoma

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Introduction: Metastatic spread of tumor cells is a highly organ specific process with the lung and liver being the most commonly affected organs. Uveal melanoma metastasis for example displays a very high selectivity for the liver. Causes and mechanisms of organ specific metastasis are largely unknown and are probably influenced by the resident endothelial cells in these organs. Liver sinusoidal endothelium (LSEC) is a prime example for organ-specific microvascular differentiation and functions. LSEC are highly specialized discontinuous and fenestrated endothelial cells that control blood plasma access to the space of Disseand as professional endocytes are responsible for clearance of the blood plasma from macromolecular waste molecules.

Results: To identify the LSEC-specific molecular differentiation program in the rat, we used a two-sided gene expression profiling approach comparing LSEC freshly isolated *ex vivo* with both lung microvascular endothelial cells (LMEC) and with LSEC cultured for 42 h. The LSEC signature consisted of 48 genes and comprised distinct sets of growth (wnt2, Fzd4, 5, 9, wls, VEGFR1, 2, 3, Nrp2) and transcription factors (Gata4, Lmo3, Tcfec, Maf) as well as endocytosis-related (Stabilin-1/2, Lyve1 andEhd3) and cytoskeleton-associated molecules (Rnd3/RhoE). In addition, our analysis identified a novel 26 kDa single-pass transmembrane protein, liver endothelial differentiation-associated protein (Leda)-1, that was selectively expressed in all liver endothelial cells and preferentially localized to the abluminal cell surface.

Furthermore, expression analysis of general EC and LSEC specific markers in murine and human hepatocellular carcinoma and melanoma metastasis revealed an endothelial expression pattern that differed in tumors as compared to the surrounding normal liver, thus facilitating identification of areas that are affected by the tumor. In tumor nodules Stabilin-2, CD32b and Lyve1 were absent and Stabilin-1 was largely reduced and only found in few endothelial cells. In contrast CD31 and Leda-1 expression was still present in endothelial cells of tumor nodules and its signal on immunohistochemistry (IHC) appeared to be stronger than in the surrounding healthy liver.

Conclusion: A LSEC-specific molecular signature could be identified by comparative gene expression profiling which might well contribute to tumor cell adhesion and establishment of metastasis in the liver. Upon cancerogenesis and metastasis the endothelial cells displayed a process of transdifferentiation (so called capillarization) which could be visualized on IHC by differences in the expression patterns of EC and LSEC marker genes.

P231
Leda-1: a novel junctional molecule expressed by the B16 mouse melanoma cell line

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Introduction: Leda-1 (liver endothelial differentiation associated protein-1) was recently identified in rat liver endothelial cells as a novel type-1 transmembrane protein that sorts to the basolateral membrane and localizes to E-Cadherin positive adherens junctions in transgenic polarized epithelial cells (MDCK). Its homologueAJAP-1 is commonly expressed in oligodendrogliomas and promotes invasiveness.

Results: An expression screening of mouse and rat tumor cell lines with a custommade guinea pig anti-rat-Leda-1 antibody revealed the B16 mouse melanoma cell line as the only tumor cell line that endogenously expressed Leda-1. Co-immunostaining with N-Cadherin revealed plasma membrane localization of Leda-1 in B16 and co-localization with N-Cadherin. Biochemical analysis revealed extensiveo-glycosylation and sialidation of Leda-1 on the extracellular surface of the plasma membrane. Furthermore extensive proteolytic processing could be demonstrated and cleavage at a furin cleavage site was identified as one step in the early processing of Leda-1.

Conclusion: Expression of the novel junctional protein Leda-1 was found and analysed in the B16 cell line. Homology to AJAP-1 suggests a role as a junctional modulator and potentially a promoter of invasiveness. Further investigations necessary to characterize the molecular and cellular functions of Leda-1 are currently in process. These include the generation of stably overexpressing and stable knock-down B16 cell lines to analyse the impact of Leda-1 on tumor progression and metastasis *in vivo* and *in vitro*.

P232
Absence of BRAF and HRAS mutations in eruptive Spitz nevi

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Eruptive Spitz nevi have been reported rarely in the literature. In solitary Spitz nevi, BRAF and HRAS mutations as well as increased copy numbers of 11p have been identified. The genetic changes underlying eruptive Spitz nevi are unknown. We report on a 16-year-old boy who developed multiple disseminated eruptive melanocytic tumours within a few months. Histopathological examination confirmed the diagnosis of Spitz nevi. We analyzed BRAF, HRAS, KRAS and NRAS genes in 39 nevi of this patient for hotspot mutations. Furthermore, comparative genomic hybridization (CGH) analysis was performed in three lesions. None of the Spitz nevi displayed a mutation in the analyzed genes, and no chromosomal imbalances were observed. Our results indicate that the typical genetic alterations described in solitary Spitz nevi appear to be absent in eruptive Spitz nevi and yet unknown genetic alterations account for this rare syndrome.

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RIP1-Tag2xSTAT1.ko mice as a tool to characterize IFN-γ dependent cancer therapy

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Transgenic RIP1-Tag2 mice express oncogenic T antigen 2 (Tag2) under the control of the rat insulin promotor 1 (RIP1) in all insulin-producing cells of the pancreas. Tag2 inhibits the tumor suppressor proteins p53, and retinoblastoma protein (Rb), leading to the development of pancreatic islet carcinomas. Previous studies revealed that Tag-specific, interferon-γ (IFN-γ)-producing T helper 1 (Th1) cells double the lifespan of RIP1-Tag2 mice. The therapeutic effect strictly depended on IFN-γ and tumor necrosis factor (TNF) signaling. One important tool for the investigation of the IFN effects was the generation of RIP1-Tag2 mice which lack the signal transducer and activator of transcription 1 (STAT1), a critical component involved in the IFN signaling cascade. STAT1-deficient mice offer no visible abnormality but display a complete lack of responsiveness to either IFN-α or IFN-γ. STAT1-knockout mice (STAT1.ko) with 129/Sv background were backcrossed for more than 10 generations to theC3H/HeJ background, and additionally, crossed with RIP1-Tag2 mice to generateRIP1-Tag2x-STAT1.ko mice. The phenotype of the islet tumors was more aggressive in the RIP1-Tag2xSTAT1.ko mice as compared with wild type islet tumors. Further, and in clear contrast to RIP1Tag2 mice, Tag-Th1 cells failed to prolong the survival of double transgenic RIP1-Tag2xSTAT1.ko mice. Within 12 weeks, the blood glucose level of Th1-treated RIP1-Tag2xSTAT1.ko mice did not differ from the sham-treated mice, indicating that both groups showed similar tumor growth. To investigate the mechanisms of the Th1-mediated therapeutic effects, we analyzed the infiltration of immune cells in the initial phase of pancreatic islet tumorigenesis. Therefore, we measured the amount of CD45 positive cells via fluorescence-activated cell sorting (FACS) in the pancreata of Th1-treated and sham-treated RIP1-Tag2xSTAT1.komice, and sorted different populations enriched in dendritic cells (DC), macrophages and natural killer cells (NK). Preliminary data revealed that the total numbers of DC in the pancreatic tissue were increased by more than 50% in Th1-treated mice compared with the sham-treated group, irrespective of the genetic background. The level of macrophages seemed to be independent of Th1 therapy or genetic background, whereas NK cells only accumulated in pancreata of RIP1-Tag2.WTmice. Taken together, the RIP1-Tag2xSTAT1.ko model enabled us to have a distinct look on the IFN signaling pathway in a model of spontaneous carcinogenesis. Furthermore, analysis of the isolated fractions of immune cells provides the basis to examine tumor dormancy induced by IFN-γ.

P234

Downregulation of AKT3 sensitizes melanoma cells to cisplatin treatment

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In melanoma, the PI3K/AKT (AKT) signaling pathway is constitutively activated and plays a decisive role in chemoresistance. However, it is not yet clear which effectors of the AKT signaling pathway determine treatment resistance in melanoma cells and which molecules are suitable molecular targets for decreasing chemoresistance. In this study, we have constructed recombinant lentivirus-delivered short hairpin RNAs (shRNAs) against components of the PI3K/AKT pathway. Using these shRNAs we tested the efficiency of downregulation of different downstream effectors of thePI3K/AKT signalling pathway on cisplatin resistance. Chemosensitivity was tested by examining the effects on growth and cell cycle of melanoma cell lines in monolayer culture. Our data show, that downregulation of AKT3, which is the dominant active form of AKT in melanoma, was able to increase cisplatin-induced apoptosis in melanoma cells up to three fold. In contrast, downregulation of single components downstream in this pathway had no significant chemosensitizing effect. These results suggest, that the PI3K/AKT signaling pathway activates several downstream targets which are involved in chemoresistance of melanoma cells and that downregulation of components upstream in this pathway could better sensitize melanoma cell towards chemotherapy.

P235

Increased number and degranulation of mast cells in cutaneous lymphomas

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Background: Primary cutaneous lymphomas (CLs) originate in the skin and are classified into cutaneous T-cell lymphomas, cutaneous B-cell lymphomas and some rare subvariants. Whereas cutaneous B-cell lymphomas usually show an indolent course, cutaneous T-cell lymphomas can be progressive and resistant to conventional therapies. Functional interactions of CLs with their tumor microenvironment are not well understood.

Objectives: Mast cells are increasingly recognized as critical regulators of the tumor microenvironment in different human malignancies. The aim of the present study was to explore the role of mast cells in the tumor microenvironment of CLs.

Methods: Skin biopsies from different CL subtypes ($n = 40$) and normal skin ($n = 6$) were evaluated by H&E staining and by immunohistochemistry with antibodies against mast cell tryptase, CD117 and CD30.

Results: The numbers of tryptase- or CD117-positive mast cells were significantly increased in skin biopsies from CL patients compared with normal skin. Mast cell infiltration was particularly prominent in the periphery of CLs at the invasive tumor front. In addition, the percentage of degranulated mast cells was significantly increased in CLs compared with normal skin. Degranulation was also mainly observed at the invasive front. CL patients with progressive forms showed higher mast cell numbers than CL patients with a stable course. There were no significant differences in the number and distribution of mast cells between cutaneous T-cell lymphomas and cutaneous B-cell lymphomas. In cutaneous T-cell lymphoma patients, maximal mast cell numbers were found in tumor stage, followed by plaque stage and eczema stage. Comparing different CL subtypes, highest mast cell infiltration was seen in folliculotropic mycosis fungoides (FMF), primary cutaneous small/medium-sized pleomorphic T-cell lymphoma (PLEO) and primary cutaneous follicle center B-cell lymphoma (CFBCL). In all CLs, areas with degranulated mast cells were associated with increased numbers of eosinophils.

Conclusions: The number and degranulation of mast cells is significantly increased inCLs, particularly at the invasive front of CLs. Mast cell counts appear to correlate with the prognosis of CL. We plan to further investigate the functional role of mast cells in the tumor microenvironment of CLs using new transgenic mouse models.

P236

Chronic TPA-mediated inflammation enhances the metastatic potential of DMBA-induced primary cutaneous melanomas in Hgf-Cdk4R24C mice

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Chronic inflammation is required for the development of carcinogen-induced papillomas in the skin in mice. Here we addressed the influence of a proinflammatory microenvironment on the growth of primary melanomas in the genetically engineeredHgf-Cdk4R24C mouse model. Cohorts of 8–10 week old Hgf-Cdk4R24C C57BL/6 mice received a single application of 100 nmol dimethyl benzanthracene (DMBA) on the shaved back skin. Half of the mice were additionally treated twice weekly by epicutaneous applications of 100 nmol 12-O-Tetradecanoylphorbol-13-acetat (TPA). DMBA-treatment led to synchronous appearance of multiple, rapidly growing nodular melanomas after 8 weeks. Mice had to be sacrificed on average 6 weeks later due to large tumor burden. All animals showed metastatic spread of melanoma cells to the draining lymph nodes and to lungs. TPA treatment promoted a strong acute inflammatory response in the skin of Hgf-Cdk4R24C mice. Furthermore a chronic TPA-mediated inflammation induced multiple papillomas but did not affect the incidence, number or growth-kinetics of primary melanomas in DMBA-exposed skin of Hgf-Cdk4R24C mice. However, we observed a significant increase in the number of lung metastases in TPA-treated mice. As a first hint towards identifying the mechanism how chronic TPA-induced inflammation can enhance the metastatic potential of melanoma cells we found a relative increase of Gr-1+ CD11b+ myeloid cells in the microenvironment of primary cutaneous melanomas. Further investigations in this experimental model may provide novel insights how chronic inflammation contributes to melanoma progression.

P237

Novel triterpenoid enriched mistletoe extracts show anti cancer effects on murine B16.F10 melanomas *in vivo*

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The European mistletoe (Viscum album L.) contains a variety of water soluble (mistletoe lectins, viscotoxins) and -insoluble (triterpenoids) substances with anti-cancer effects. This makes mistletoe derived extracts and compounds interesting for treatment of cancers with low response rates like melanoma. Mistletoe therapy is one of the most important complementary therapies in Central Europe but up to date the standard preparations contain only water soluble substances of the plant. Regarding the literature, triterpenoids and their derivatives show anti cancer effects on melanoma and other cancer cell lines, which were mainly limited to the *in vitro* setting because most triterpenoids are water insoluble. Triterpenoid extracts from mistletoe (80% oleanolic acid and 4% betulinic acid) are well characterized and solubilization with 2-hydroxypropyl-beta-cyclodextrin makes them available for cell culture experiments and cancer treatment in animal models as so called solubilized triterpene extracts (STE).

In our *in vivo* studies B16.F10 melanoma cells were inoculated subcutaneously (sc) into the flanks of C57BL/6 mice. Treatment with mistletoe extracts (sc injections) were started three days after tumor inoculation for 10 cycles (every second day). The tumor size was determined by calliper measurement every second day.

High dose mistletoe treatment displayed anti cancer effects by slowing down melanoma growth and increasing survival of B16.F10 melanoma bearing mice. While standard mistletoe extracts (containing mistletoe lectins) show only moderate effects on tumor growth, mistletoe extracts enriched with STE evoke increased effects by inducing tumor regressions. Histological examinations of the tumors and surrounding tissue show, that mistletoe extracts induce tumor necrosis, moderate caspase-3 activation and are also able to decrease neoangiogenesis compared to control animals. Mistletoe extracts enriched with STE cause higher caspase-3 activation than standard mistletoe extracts.

We demonstrate here, that standard mistletoe extracts have anti-cancer effects onB16.F10 melanoma *in vivo*. Interestingly, novel mistletoe preparations containing STE from mistletoe in addition to water soluble mistletoe lectins develop improved anti-cancer effects.

P238 (V35)

Role of WEE1 in cell cycle regulation of malignant melanoma

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Tumor suppression pathways are often compromised in malignant tumors, with the most prominent example being p53. p53 is often mutated and thereby inactivated in tumors of epithelial origin. However, the p53 pathway also appears to play a role in malignant melanoma via as yet poorly understood mechanisms. We were able to demonstrate that 14-3-3σ, a well-known downstream target of p53 is inactivated in malignant melanoma by epigenetic silencing (gene methylation). In line with this, demethylation of the 14-3-3σ gene with consecutive overexpression induced cell cycle arrest in melanoma cells, an effect that could almost completely be abolished by 14-3-3σ knockdown. In our recent experiments, we were interested in the downstream molecules that might mediate the regulatory effects of 14-3-3σ on cell cycle. We analysed the role of WEE1, a recently described putative interaction partner of 14-3-3σ, which was identified in a proteomic screen after affinity purification by another group. WEE1 is a well-known signaling kinase of the G2/Mcheck point of cell cycle, controlling mitotic entry. Indeed, we could demonstrate by co-immunoprecipitation assays of melanoma cell extracts that WEE1 interacts with14-3-3σ. Other putative interaction partners of 14-3-3σ involved in cell cycle control such as c-TAK1 and AJUBA did not interact with 14-3-3σ in our analyses. Moreover, 14-3-3σ induction after gene demethylation in melanoma cells was followed by nuclear translocation of WEE1. Interestingly, treatment of melanoma cells with classical cytostatic agents reduced cytoplasmic and nuclear WEE1 expression (which has also been described in other tumors). Taken together, nuclear translocation and activation of WEE1 might explain the cell cycle inhibitory effects of 14-3-3σ after gene induction by demethylating agents. Based on these findings, demethylating agents might be interesting therapeutic options for malignant melanoma in the future.

P239

Trogocytosis of melanoma

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Several reports have documented that lymphocytes can extract surface molecules through the 'immunological synapse' from the antigen-presenting cells (APC). This phenomenon was termed 'trogocytosis' (from the ancient Greek trogo, meaning 'gnaw') and is a mechanism of fast, cell-to-cell contact-dependent uptake of membranes and associated molecules from one cell by another. Trogocytosis could be a vector for intercellular communication and manipulation, as molecules acquired during the transfer endow the acceptor cell with some functions of the donor cells and therefore might alter or regulate their activity status. Trogocytosis is not restricted to the interaction between APC and T cells, but also has been documented in monocytes, B cells and natural killer cells both *in vitro* and *in vivo*. Thus, this process may be important in the induction and regulation of immune responses, and possibly in the control of other cellular systems.

At the site of melanoma, inflammation with leukocyte infiltration is regularly observed, but infiltrating leukocytes hardly have long lasting tumoricidal effects. Whether trogocytosis between melanoma cells and leukocytes and subsequent changes in the phenotype of the latter occur, has not yet been analyzed.

Here we investigated the transfer of specific endogenous surface proteins of different melanoma cell lines to resting PBMC in co-culture experiments *in vitro*. Flowcytometric analysis after 3 h of co-culture revealed that up to 40% or 90% of CD14+ monocytes and 10% or 25% CD4+ T cells had

acquired the melanoma specific molecules MCSP or MCAM respectively, while the efficacy depends on the melanoma cell line used. Activation of monocytes with PMA before co-culture further increased especially the transfer of MCSP. Furthermore, *ex vivo* isolated melanoma-derived CD14+ and CD4+ cells carried MCSP, but CD14+ cells showed amore intense MCSP signal. So far we demonstrated that resting, and even more so, activated CD14+ monocytes and CD4+ T cells efficiently acquired different membrane-bound melanoma specific surface molecules by trogocytosis *in vitro* and *in vivo*.
Our data highlight for the first time the possible role of membrane-bound molecules that can be trogocytosed by leukocytes in the melanoma microenvironment. Yet we can hypothesize that melanoma cells might transfer immuno-modulatory molecules onto activated immune cells via trogocytosis, and thereby modulate their activatory or regulatory functions, resulting in an efficient immune escape mechanism.

P240
Regulation of Brn3a in melanoma

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Melanoma is – due to its resistance to therapy in advanced stages – the skin cancer with the highest mortality. We have previously shown that the neuronal transcription factor Brn3a is highly expressed in human melanoma cells, but not in primary cells of the skin such as fibroblasts or melanocytes. Expression was confirmed in primary melanoma biopsies, whereas benign melanocytic nevi were found to be negative. Importantly, inhibition of Brn3a results in cell cycle arrest followed by apoptosis in melanoma cell lines and reduces tumor growth *in vivo*. These results suggest a crucial role of Brn3a in melanoma, however the factors that lead to upregulation of Brn3a are not known.
Brn3a was upregulated on RNA and protein level. To characterize the transcriptional regulation, we analyzed the 5' region of the Brn3a gene. For this, we cloned the proximal 4.5 kb region upstream of the translational start site of the Brn3a gene in front of a luciferase reporter gene. Confirming the observation regarding Brn3alevels, reporter activity was restricted to the melanoma cells, whereas no reporter signal was observed in Brn3a-negative primary fibroblasts. To characterize the promoter region in more detail, we performed targeted deletions of the 4.5 kb region and assessed the reporter activity. It was found that the 1.5 kb and a 1 kb region upstream of the translational start site displayed a similar reporter activity. This indicates that the Brn3a promoter region is located within the 1000 bp region.
We are currently analyzing this region to identify transcription factors that are responsible for Brn3a upregulation. Due to the crucial role of Brn3a, identified transcription factors may represent novel therapeutic targets for melanoma.

P241
Ultraviolet A radiation plays a role in the pathogenesis of malignant melanoma through involvement of the Warburg effect in skin reconstructs

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Malignant melanoma is a highly aggressive skin tumor. While recent studies elegantly demonstrated a causative role for a single high dose of ultraviolet (UV) B (280–320 nm) radiation in the development of melanoma, the role of UVA (320–400 nm) in the pathogenesis of human melanoma remains unclear.
We could previously show, that repetitive exposure of melanoma cell lines to UVA irradiation increases lactate levels and increases levels of the transketolase-like-1 enzyme, which is an important enzyme of the pentose phosphate pathway. These findings reason for an increase of aerobic glycolysis after repetitive UVA exposure. This phenomenon is characteristic for many carcinomas and is known as the Warburg effect.
To gain more insight in the role of UVA in the pathogenesis of melanoma it is important to focus investigations on model systems resembling the human skin better than single cell cultures. To address this issue, we constructed *in vitro* models of human skin, either employing melanocytes or melanoma cells of different malignancies and exposed them to sublethal, repetitive UVA irradiation. During repetitive UVA irradiation, levels of lactate and glucose were measured and upon completion of UVA treatment transketolase activity and the levels of ß Galactosidase associated senescence (ß-Gal) was measured.
We found, that UVA influences glucose consumption and lactate production in skin models with melanocytes and melanoma cell lines. Furthermore, UVA elevates transketolase activity in melanocytic skin models compared to melanoma skin models. In addition to this, UVA alters ß-Gal. These findings support the hypothesis that repetitive doses of UVA may also play a role in the pathogenesis of melanoma.

P242
Metastatic melanoma cells are sensitive to drugs inducing endoplasmic reticulum stress-mediated apoptosis

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Both the RAF-MEK-ERK and PI3K-AKT-mTOR signaling pathways have been reported to be relevant to melanoma progression and drug resistance. Intriguingly, a recent phase I study showed that PLX4032, a selective inhibitor for BRAFV600E kinase, led to significant remission in nine out of 16 patients with metastaticBRAFV600E melanoma demonstrating that selective potent inhibition of a relevant altered signal transduction molecule may be effective in selected patients with metastatic melanoma. Furthermore, recent experimental studies suggest that in melanoma combined inhibition of both signaling pathways is also a promising treatment strategy. We observed that combinations of the pan-RAF inhibitors sorafenib or RAF265 with the mTOR inhibitors sirolimus or RAD-001 significantly inhibit growth, potently induce apoptosis and almost completely suppress invasive growth of metastatic melanoma cells in monolayer and organotypic culture, respectively.
To obtain insight into the mechanisms by which these inhibitors exert antitumor activity, we analysed the gene expression profile. Microarray analysis showed upregulation of a series of genes (p8, CHOP, ATF4, ATF3 and TRB3) that are known to be involved in endoplasmic reticulum stress-mediated apoptosis. Real-time quantitative PCR confirmed that combinations of pan-RAF inhibitors with mTOR inhibitors upregulate p8, CHOP, ATF4, ATF3 and TRB3 mRNA levels. Furthermore, the BRAFV600E kinase inhibitor PLX4032 inhibited growth and upregulated CHOP, ATF4, ATF3 and TRB3 mRNA levels exclusively in BRAFV600E mutated melanoma cell lines. Moreover, classical endoplasmic reticulum stress inducers such asthapsigargin potently inhibited growth, induced apoptosis and upregulated p8 mRNAlevels in all metastatic melanoma cell lines tested.
These data suggest that metastatic melanoma cells are sensitive to drugs capable of inducing endoplasmic reticulum stress-mediated apoptosis.

P243
Beta-catenin has a central role in melanoma progression

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Since more than two decades the importance of beta-catenin in melanoma progression is a matter of debate. Recently, we found that the expression of casein kinase 1alpha EUR'a kinase phosphorylating beta-catenin and thus inducing its degradation- is down-regulated in metastatic melanoma cells, suggesting an important role of beta-catenin during melanoma progression (Sinnberg et al. 2010).
To analyze the impact of beta-catenin on melanoma progression, we downregulated beta-catenin expression and activity using either a small molecule beta-catenin inhibitor (PKF115-584) or a specific shRNA against beta-catenin. Viability, proliferation, invasion and metastatic capability of melanoma cells of different progression stages including benign melanocytes were assayed for their sensitivity to beta-catenin inhibition. Interestingly, we found a diminished proliferation rate, reduced invasive capability and induction of apoptosis in metastatic melanoma cells, whereas early growth phase melanoma cells were less affected. Primary melanocytes were completely unfazed by beta-catenin inhibition. The strong apoptosis induction in metastatic melanoma cells indicates that beta-catenin is an essential survival factor in late-stage melanoma cells and plays an important role in melanoma progression.

P244 (V28)
PI3K and MAPK signaling activate the transcription factor YB-1 promoting chemoresistance and invasive tumor growth

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We have recently shown that YB-1 is a transcription factor influencing melanoma cell proliferation, invasion, apoptosis induction as well as chemoresistance in melanoma cell lines. So far, nothing is known about the regulatory pathways that influence the activation of YB-1 in melanoma. From mammary tumors it is known that phosphorylation of YB-1 on Ser102 is promoting nuclear translocation and transcription factor activity on the promoter of YB-1 target genes. To identify the signaling pathways involved in YB-1 activation in melanoma, we established a reporter system by which we could monitor the activity of the YB-1 promotor. Inhibition of the PI3K/AKT signaling pathway using wortmannin or LY294002significantly inhibited YB-1 promoter activity, whereas inhibition of the MAPK signaling pathway had no effect. The specificity of the signal transduction inhibitors were confirmed using siRNA against Erk1/2 for the MAPK signaling and AKT3 forPI3K/AKT signaling. Furthermore, we have identified the factors and signalling pathways that are involved in phosphorylation and nuclear translocation of YB-1. We found that the PI3K and the MAPK signalling pathways are essential for YB-1 Ser102 phosphorylation and nuclear translocation whereas NF-kappa B signalling is inhibitory. These data indicate that the PI3K/AKT signaling pathway activates theYB-1 promotor and Ser102 phosphorylation, whereas the MAPK signaling pathway is able to activate YB-1 phosphorylation but not the promoter activity. The amount of YB-1 expression, Ser102 phosphorylation and nuclear translocation was measured inhuman patient material, the HGFxCDK4 (R24C) melanoma mouse model as well as in the Xmrk melanoma platy fish model. These data reveal a further mechanism by which the MAPK and PI3K pathways mediate chemoresistance and invasive growth of melanoma cells, namely by the activation and induction of YB-1 transcriptional activity.

P245
Impact of extracellular matrix components on activation of the PI3K/Aktsignaling pathway and on chemoresistance in melanoma

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Constitutive activation of the PI3K/Akt signaling pathway as well as the tumor microenvironment are involved in enhanced chemoresistance of melanoma cells.
In this work the impact of several extracellular matrix molecules and soluble factors of the native tumor microenvironment on chemoresistance and activation of PI3K/Aktsignaling in melanoma cells was analyzed. For this purpose, the metastatic melanoma cell lines SKMel-28 and 451-LU were cultured on Collagen I, Laminin, Fibronectin or Poly-L-Lysine or treated with soluble factors from the tumor microenvironment. After treatment with cisplatin the viability of the cells was measured and activation of the PI3K/Akt signaling pathway was investigated using western blot analysis of phosphorylated Akt. Furthermore, immunofluorescence analysis was used to elucidate if three-dimensional cultivation of melanoma cells with fibroblasts causes changes in the intracellular distribution of activated Akt. Our results show that Collagen I, Laminin and Poly-L-Lysine were able to increase chemoresistance of melanoma cells towards cisplatin without stimulating the phosphorylation of Akt. Interestingly, melanoma cell attachment to the extracellular matrix molecules attenuated cisplatin induced activation of the tumor-suppressor p53, representing a possible mechanism responsible for the increased chemoresistance. Soluble factors from the tumor microenvironment strongly enhanced chemoresistance especially when cells were plated on Collagen I matrix. Furthermore, interaction of SKMel-28 with fibroblasts resulted in translocation of activated Akt into the nucleus. In summary, our data indicate that extracellular matrix molecules and soluble factors from the tumor microenvironment enhance chemoresistance of melanoma cells, partially by suppression of p53 activation and nuclear translocation of phosphorylated Akt.

P246 (V07)
Bone morphogenetic protein and nodal regulate adhesion and migration in melanoma cells and confer a malignant phenotype to melanocytes *in vitro* and *in vivo*

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Embryonic signalling is reactivated during malignant transformation in melanoma. During embryonic development, TGF- family members nodal and bone morphogenetic protein-2 (BMP-2) induce an epithelial-mesenchymal transition (EMT) in the epiblast and in the neural crest, respectively. We previously showed that the BMP-antagonist noggin blocks both physiologic (EMT) and invasive migration of melanoma cells in the neural crest and the optic cup of the chick embryo. Here we demonstrate numerous effects of the agonists BMP-2, BMP-7 and nodal, and their antagonists noggin and lefty, and the nodal receptor antagonist SB431542 on melanoma cells and melanocytes.
Neither agonists nor antagonists had an effect on cell cycle or cell proliferation. Melanoma cell adhesiveness assessed by primary aggregate formation was reduced by agonist treatment and increased by antagonists. Migration and invasion were increased by agonists and blocked by antagonists. The effects on adhesiveness were confirmed for up to 3 weeks after a single treatment with the recombinant human proteins. Western Blot analyses showed an up-regulation of neural crest-specific proteins Slug and SOX9 upon agonist treatment and their down-regulation by antagonists in melanoma cells. Further, mTOR signalling, constitutively activated in melanoma cells, was inhibited by antagonists. Real-time PCR analyses showed alterations in TGF – related genes upon treatment. In the epidermal skin reconstruct invasive migration of melanoma cells was reduced by antagonists. *In vivo*, nodal antago-

nists also inhibited neural crest migration (EMT) of SKMel28 melanoma cells upon transplantation into the chick embryonic neural tube.
Agonists (BMP-2, BMP-7, nodal) conferred a malignant phenotype to benign (verified by comparative genomic hybridization and exclusion of BRAF and NRAS-mutations) primary human melanocytes: They reduced adhesiveness, induced migration and invasion *in vitro*, and altered neural crest-specific proteins and expression of TGF – related genes. In addition, they induced mTOR signalling. *In vivo* BMP-2 and nodal induced EMT and integration of transplanted melanocytes into the chick embryonic neural crest, thus behaving like untreated melanoma cells. Untreated melanocytes failed to integrate into the neural crest *in vivo* (like antagonist-treated metastatic melanoma cells).
To underline the clinical importance of these findings, expression of BMP- and nodal receptors, as well as a large panel of (BMP-downstream) neural crest-specific proteins was determined for the first time in a newly-generated tissue microarray consisting of samples of 307 melanocytic lesions accompanied by clinical follow-up data (between 10 and 17 years) of the German Melanoma Registry. Finally, the influence of BMP-2 and nodal (both secreted by melanoma cells) on murine dendritic cells was assessed.
In summary, we highlight that BMP and nodal are crucial for melanoma cell invasiveness *in vitro* and *in vivo*. Moreover, we show for the first time that treatment with only one single protein (BMP-2, BMP-7, nodal) is sufficient to confer amelanoma phenotype to benign primary melanocytes *in vitro* and to induce an EMT of such pre-treated melanocytes *in vivo*.
Together, we are able to demonstrate that BMPs and nodal represent highly crucial therapeutic targets to prevent the spreading of primary melanomas.

P247
Mechanisms of cytotoxic effects of ascorbate on melanoma cells

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Despite its controversial history in cancer therapy, a large body of evidence emerged in recent years supporting the original hypothesis of Linus Pauling that high doses of ascorbate exert cytotoxic effects on numerous cancer cells *in vitro* and *in vivo*. In clinical trials high-dose i.v.-ascorbate therapy in cancer patients showed an excellent tolerability without severe adverse events; series of case reports demonstrated a prolonged survival of stage IV cancer patients after high-dose ascorbate treatment.
Here we report that ascorbate bears numerous and diverse cytotoxic effects on melanoma cells. These effects are driven by copper-dependent formation of ascorbate radicals and hydrogen peroxide (H2O2) with subsequent interference with cell metabolism (reduction of ATP, glutathione, and NADPH levels), time-dependent increase of the sub-G1-fraction in cell cycle analyses, alteration of miRNA expression, complete degradation of mitochondria with cytochrome c release, dose-dependent inhibition of basal autophagy, and induction of apoptosis eventually leading to DNA cleavage and necrosis within 24 h after treatment. mRNA and protein array analyses of melanoma cells consistently confirmed an up-regulation of redox equivalents and immune response (IL6 and IL8), and a down-regulation of genes involved in energy metabolism and chromatin integrity. Histone deacytelases were not significantly inhibited by ascorbate. High doses of ascorbate were selectively toxic for melanoma cells when compared to 12 different somatic and stem cell populations.
To extend the results gained on well-characterized melanoma cell lines that have been cultured for decades, we pursued an additional *ex-vivo* approach, in which melanoma cells were isolated from skin metastases of 15 consecutive melanoma patients and kept in short-term cell culture to mimic a clinical therapeutic setting. Chemosensitivity arrays of such patient-derived metastatic melanoma cells showed significantly enhanced cytotoxic effects of all chemotherapeutics tested upon combination with ascorbate, which also enhanced the effects of repetitive –radiation on melanoma cells. *In vivo*, high-dose ascorbate administered intraperitoneally reduced melanoma metastasis formation in mice after i.v. injection of melanoma cells.
To further demonstrate a clinical significance, ascorbate levels were determined in serum of 120 melanoma patients and in 46 primary melanocytic tissues. Moreover, expression of ascorbate-regulated proteins (HIF-1, GLUT-1) was determined in a newly-generated tissue microarray consisting of samples of 307 melanocytic lesions accompanied by clinical follow-up data (between 10 and 17 years) of the German Melanoma Registry.
Together, we show for the first time the vast extent of the complexity and diversity of cytotoxic mechanisms of ascorbate on melanoma cells and its clinical impact, and conclude that high-dose ascorbate is an

P248 (V27)
RAGE activity relates to melanoma clinical stages and progression

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In addition to tumor cell-intrinsic mechanisms, melanoma initiation, growth and progression have been related to microenvironmental factors orchestrating tumor-stroma interaction. However, the mechanisms that sustain a tumor-promoting micro-environment remain largely elusive, especially in malignant melanoma.
We have recently demonstrated that the receptor for advanced glycation end-products (RAGE) is central for mediating experimental non-melanoma skin tumor formation as well as for experimental chronic inflammation by sustaining positive signaling feed-forward loops regulating specific sets of pro-inflammatory genes such as certain chemokines, COX-2, TNF-a and IL-6.
Here, we describe that RAGE activity relates to human melanoma clinical stages and progression and therefore might be central in regulating melanoma growth and development. Markers of RAGE activity include RAGE protein expression, serum levels of a soluble form of RAGE (sRAGE), phosphorylation of cellular down-stream targets such as transcription factors NF-kappaB p65, Jun and Stat3 as well as protein expression of RAGE targets/activating ligands such as S100A8/A9, S100B, HMGB1 in human melanoma specimens ($n = 124$). As determined by immunofluorescence on human melanoma tissue sections RAGE protein expression is up-regulated in a stage-dependent manner; by using sRAGE specific ELISA levels of sRAGE are significantly down-regulated in the serum of melanoma patients at stage III compared to patients at stage I and II. Moreover, sRAGE serum levels are significantly down-regulated in patients at stage IV compared to any other stage. Activity of p65, Junand Stat3 as well as protein expression of S100A8/A9, S100B, and HMGB1 as determined by a combination of immunofluorescence and ELISA on human melanoma tissue/serum specimens correlated conversely in a stage-dependent manner. These findings in humans are at least partly resembled in mice by using MT/ret transgenic and transplantation melanoma mouse models as well as RAGE-deficient mice.
In conclusion, we provide multiple evidence for a novel role of RAGE signaling in driving melanoma growth and development by highlighting the importance of melanoma-stroma interaction in a RAGE-dependent manner. Moreover, we shed light on RAGE signaling as a novel clinical marker as well as a promising target for anti-melanoma therapy.

P249
Slug augments the epithelial-mesenchymal like transition in melanoma through transcriptional activation of ZEB1

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Epithelial – Mesenchymal Transition (EMT) is an important step in tumour development and describes a process that is accompanied by fundamental molecular changes causing a loss of epithelial and a gain in mesenchymal characteristics. Many of these changes are subject to the control of a few transcription factors known as EMT regulators (EMTRs), including the zinc finger transcription factors Slug andZEB1 and helix-loop-helix transcription factors like Twist. Both Slug and ZEB1 have been described as modulators of cell-cell adhesion and migration, repressing the expression of the adhesion molecule E-cadherin.
Here we report that ZEB1 is activated at the transcriptional level by Slug. Lentiviral overexpression of Slug in WM9 or WM164 melanoma cells is followed by upregulation of ZEB1, both at the mRNA and protein level, whereas silencing of Slug leads to the reverse effect. Four potential target sequences (E-boxes) for Slug were identified at the ZEB1 promoter in a region from -3000 to +200 relative to the transcriptional initiation site. Gel shift assays revealed binding of Slug to all four of these E-boxes with different affinities. Luciferase assays confirmed that Slug is initiating transcriptional activity at the ZEB1 promoter. The effects of Slug on ZEB1 promoter regulation are specific, since Snail is not binding and Twist only binding with low affinity in gel shift assays, but Twist does not lead to significantly enhanced luciferase activity. Further, Slug and ZEB1 cooperatively regulate E-cadherin expression and the effect of both EMTRs on cell-cell adhesion and cell migration is additive. These data suggest that a hierarchical and cooperative sequence inactivation of EMTRs results in sustainable changes of the epithelial phenotype in melanoma.

P250 (V03)
cIAPs block TLR3-mediated cell death by interference with Ripoptosome formation, a novel RIP1/caspase-8 containing intracellular complex

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Signals activated by ligation of innate immune receptors such as Toll-like receptors (TLRs) are of major importance for the skin immune system and represent the first 'interaction border' against pathogens or allergens. Moreover TLR-induced signals influence qualitative and quantitative immune responses in the skin. In this report, we have found that cellular inhibitors of apoptosis proteins (cIAPs) can act as inhibitors of Toll-like receptor-3 (TLR3)-induced signalling pathways as exemplified by their negative regulation of TLR-3-induced cell death. We demonstrate that loss of cIAPs profoundly modifies the response to the mimic of the natural TLR-3 ligand double stranded RNA (poly (I:C)). Poly (I:C) induced dramatic cell death in both a caspase and RIP1-kinase-dependent manner. Loss of cIAPs in keratinocytes induced either by IAP antagonists or by stable cIAP1, cIAP2, or cIAP1 and cIAP2 knockdown resulted in sensitization to cell death. Using coimmuno-precipitation under native endogenous expression conditions, we found that in the absence of cIAPs, the spontaneous formation of a novel thus far unknown intracellular complex, that we designate the 'Ripoptosome', is detected. This intracellular protein complex is necessary, but not sufficient for cell death induction and contains caspase-8, cFLIP, FADD and RIP1. Upon TLR3 ligation, this complex is recruited to the adaptor protein TIR-domain-containing adapter-inducing interferon-β (TRIF) in a stimulation-dependent manner. Interestingly the caspase-8 inhibitor cFLIPL but not cFLIPS conferred substantial protection from IAP antagonist or TNF-like weak inducer of apoptosis (TWEAK)-induced degradation of cIAPs and subsequent formation of the Ripoptosome. These data implicate cIAPs as important negative regulators of this complex. The detected deviation of TLR3-mediated cell death from apoptosis to a necrosis form of cell death at the Ripoptosome that is blocked bycIAPs and activated by TLR3 ligation, CD95, and possibly other signaling pathways may have important pathophysiological consequences during inflammatory responses in the skin. Moreover modulation of the quality of cell death responses by regulation of cIAPs might impact the tumor immune response, thereby facilitating efficient tumor elimination.

P251
Antitumoral efficacy of low temperature plasma against malignant melanoma cells *in vitro*

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Introduction: In the last years plasma has been demonstrated to influence cells (induction of cell growth but also of apoptosis and necrosis), kill microorganisms and disinfect skin. Current developments in plasma-medicine include dental applications, wound disinfection, skin modelling, sterilization of medical products and implant surface bioengineering. Since *in-vitro* plasma irradiation has proven potent induction of apoptosis of glioma and melanoma tumor cells without harm of non malignant tissue cells, plasma could play a role in the treatment of skin melanoma treatment. Therefore we investigated the potency of two different plasma sources to induce apoptosis in melanoma and additional Burkitt- lymphoma and glioblastoma cells *in vitro* in order to dimensionate further therapy of mice melanoma *in vivo* (B16).
Methods: The B16 mice melanoma cells were irradiated with a dielectric barrier discharge plasma (DBD) in ambient air and an atmospheric pressure plasma jet (APPJ) using Argon as feeding gas. The cells were grown in micro well-plates and irradiated for different time intervals (30 s–2 min). After irradiation the viability, cytotoxicity and apoptosis were measured after 24, 48 and 72 h.
Results: Ninety seconds plasma irradiation using DBD or APPJ provoked a marked induction of apoptosis in melanoma cells measurable 48 h after treatment. The viability of cells showed a decrease 72 h after treatment.
Conclusion: We conclude a treatment of at least 90 s to be necessary for *in vivo* treatment of malignant melanoma metastases in mice.

P252 (V17)
Role of Rac1 in intercellular communication within the epidermis

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Objectives: Stratified human epidermis consists of multiple layers of keratinocytes that are maintained by stem cells, which have the capacity of self-renewal, and their progeny, the transit amplifying cells. In squamous cell carcinoma (SCC) or hyper proliferative skin diseases (Psoriasis), the organisation of these stratified layers of keratinocytes is disrupted and integrin expression, normally confined to the

basal layer is abnormally expressed by suprabasal cells. Terminally differentiating cells can communicate with the basal cell compartment including the stem cells to stimulate or inhibit expansion of mutant stem-cell clones involved in the earliest steps of skin carcinogenesis as shown by two-stage chemical carcinogenesis experiments. Rac1, a small GTPases of the Rho-superfamily, which has been found upregulated inSCCs, relays signals downstream of integrins to the cytoplasm and is involved in the regulation of cell movement, polarity, adhesion, gene transcription, cell cycle progression and enzyme activity.
Methods: One way to study basal-suprabasal communication is by using mouse models which express genes of interest under the control of the involucrin promoter targeting specifically terminally differentiated cells. We created a mouse model expressing eGFP together with Rac1 QL, an active form of Rac1 under the control of the involucrin promoter. We analyzed this mouse model using immunofluorescence, electron microscopy, gene expression and stratification experiments of differentiated cells overlying basal cells transfected with a Smad2/3-luciferase reporter.
Results: Rac1 overexpression by differentiated cells lead to epidermal acanthosis, hyperkeratosis, parakeratosis, hypergranulosis, spongiosis and mild dermal lymphatic infiltration. At the age of >8 months spontaneous tumour formation was observed in about 0.2% of transgenic mice including squamous cell carcinoma (from moderate differentiated SCCs to spindle cell carcinoma) and papilloma. Increase of cell colonies in number and size were demonstrated by colony forming assay using feeder cells. Cell growth was enhanced and expansion of Keratin 14 positive basal cells were noticed. Transmission electron microscopy showed strong increase in number of desmosomes, especially in the basal-suprabasal zone, clumped keratin filaments as well as strong interaction with the basement membrane. Several members of desmosomes were uregulated including desmoplakin, periplakin anddesmocollin-2. Stratification with TG but not WT differentiated cells pertubatedSmad2/3-responsiveness to TGFβ
Conclusion: Suprabasal expression of the small-GTPase Rac1 using a mouse model expressing active Rac1 driven by the involucrin promoter has a clear effect on epidermal formation: it is leading to hyper proliferation and finally to spontaneous tumour formation, clonal expansion of basal cells which exit the basal layer and move to suprabasal areas. Downstream effectors of Rac1 might be affected by ROS levels as Rac1 is part of the NADPH-oxidase complex NOX2/pg91phox, the most relevant in skin and keratinocytes. Further experiments will a how ROS levels may interfere with adherent junction formation or TGF-β responsiveness.

P253 (V02)
T cell mediated immune surveillance promotes melanoma cell de differentiation in the genetically engineered Hgf-Cdk4R24C mouse model

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Based on clinical observations it has been hypothesized that cellular immune responses play an important role in the natural progression of primary malignant melanoma in the skin. We experimentally addressed this hypothesis in the genetically engineered Hgf-Cdk4R24C mouse melanoma model using an adoptive lymphocyte transfer and vaccination approach to enforce cellular immune surveillance. Hgf-Cdk4R24C mice spontaneously develop large numbers of melanocytic nevi and single primary cutaneous melanomas with high penetrance during their first year of life. Primary cutaneous Hgf-Cdk4R24C melanomas grow progressively and metastasize in the draining lymph nodes and visceral organs. Adoptively transferred and *in vivo* activated melanoma-specific T cells are able to destroy autochthonous primary tumors. However, some tumor cells are able to survive, evade immune cell control and recur both locally and systemically. Interestingly, we observed large hypo- and amelanotic tumor areas in 40% of recurring melanomas. These amelanotic tumor areas were found in only 5% of untreated melanomas. We further investigated the mechanism of tumor immune evasion using the HCmel 384 cell line derived from a primary cutaneous Hgf-Cdk4R24C melanoma. This cell line grows progressively after subcutaneous injection in immunocompetent syngeneic C57BL/6 mice and recapitulates the histo morphology of primary tumors. Established HCmel348 melanomas regress after adoptive transfer and *in vivo* activation of melanoma-specific T cells but frequently recur several weeks later. These recurring tumors also show a considerable increase in hypo- and amelanotic tumor areas. Taken together, these results provide clear evidence that T cell mediated immune surveillance can actively shape the pathogenesis of melanoma and promote the development of dedifferentiated tumor cell subpopulations in this experimental system of primary autochthonous as well as transplanted mouse melanomas. We arecurrently investigating this phenomenon in greater detail to unravel the underlying mechanisms and understand the importance for tumor immune escape.

P254
MMP-9 supplied by bone marrow-derived cells does not contribute to melanoma lung metastasis

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Melanoma has a high probability of metastasizing to the lung. The matrix metalloproteinases (MMPs) are a family of proteolytic enzymes including more than24 human MMPs, that contain a zinc ion in the active site. MMPs function in physiological and pathological processes including migration, angiogenesis, and tissue repair, as well as metastasis and tumor progression. Up to now it is unclear whether MMP-9 secreted from infiltrating bone-marrow derived cells or resident cells in the lung contribute to pulmonal melanoma metastasis.
Using a tail vein injection experimental lung metastasis model, MMP-9 knockout mice demonstrated a more than two-fold decrease in melanoma lung metastasis compared to wild type mice following injection with B16F10 syngeneic melanoma cells. There constitution of the bone marrow of MMP-9 knockout mice with bone marrow competent to produce MMP-9 did not recapitulate the WT phenotype of overwhelming burden of pulmonal metastasis. In contrast, wilde type mice reconstituted with the bone marrow of MMP-9 knockout mice displayed the same burden of pulmonal metastatic disease as the WT phenotype. Hence, these results demonstrate that rather stromal derived MMP-9 from resident cells contribute to melanoma lung metastasis instead of MMP-9 secreted from infiltrating bone-marrow derived cells. These results provide new insights into the influence of MMP-9 in melanoma lung metastasis and demonstrates that bone-marrow derived MMP-9 is not essential for the growth and establishment of pulmonal melanoma metastasis.

P255 (V25)
Epigenetic profiling of lymphoma reveals a distinctive signature for mediastinalgray zone lymphoma

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The pathogenesis and therapy of many lymphoma subtypes of skin and lymph nodes are still challenging. Recent studies have identified epigenetic changes as a key component of carcinogenesis. Here, we studied the methylation profile of mediastinal gray zone lymphoma (MGZL), a newly recognized entity that demonstrates transitional morphologic and phenotypic features between classical Hodgkin's lymphoma, nodular sclerosis subtype (CHLNS) and primary mediastinal large B-cell lymphoma (PMBL). CHLNS and PMBL differ in morphology, immunophenotype, and therapeutic consequences. MGZLs present a challenge both to the pathologist and clinician, as the criteria to distinguish MGZL from CHLNS and PMBL are still imprecise, and the optimal treatment approach is as yet undetermined. By performing a large scale DNA methylation array of MGZL, CHLNS, and PMBL as well as diffuse large B-cell lymphoma (DLBCL) we investigated the biological underpinnings of MGZL and how it corresponds to the two related entities CHLNS and PMBL and the less related entity DLBCL. Principal component analysis demonstrated that MGZLs have a distinct epigenetic profile intermediate between CHLNS and PMBL but clearly different from that of DLBCL. Based on their epigenetic profiles we were able to establish class prediction models that could distinguish between MGZL, CHLNS and PMBL with a final combined prediction of 100%. Pyro sequencing for selected CpG sites from different genes were performed and confirmed the accuracy of the Illumina Golden Gate Methylation array results. In summary, MGZLs share several clinical and pathological features with CHLNS and PMBL. Our findings further underscore the close biological relationship between MGZL, CHLNS and PMBL, and ready distinction from DLBCL. However, MGZL has a distinct epigenetic identity that shares elements of both parent disorders. As the first biological study on MGZL, our results provide novel insights into MGZL pathogenesis, and its relationship to CHLNS and PMBL.

P256
Functional characteristics of textiles equipped with cyclodextrin-antiseptics-complexes

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Introduction: Textile materials are used in basic applications both in the cosmetics and pharmaceutical industries, e.g. tissues are utilized as carriers for active pharmaceutical ingredients and fabrics coated with antimicrobial agents are employed in the treatment of chronic wounds. Hence, we have tested the equipment of textiles with cyclodextrin-agent-complexes as a new dosage form of antimicrobial substances. Cyclodextrins (CD) are ring-shaped degradation products of starch. The most important CDs are composed of 6, 7, or 8 glucose molecules and are named α-Cyclodextrin, β-Cyclodextrin und γ-Cyclodextrin. For antimicrobial substances such as iodine (IOD), polihexanide (PHMB), and chorhexidine diacetae (CHX) the packaging into CDs could achieve a better skin compatibility, higher antimicrobial activity, and increased storage stability. We have examined the effect of the CD-antisepticacomplexes on the proliferation of human cells using a HaCa Tkeratinocyte model. Furthermore, the antimicrobial activity of textiles equipped with the CD-antiseptics-complexes against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* as model organisms was analyzed.
Material & Methods: Concentration and time dependent effects on human HaCa Tkeratinocytes were determined by chemiluminescent measurement of the cellular ATP content (ATP Lite TM-M, Perkin-Elmer). Furthermore, the release of the cytokines IL 6 and IL 8 was assessed by specific ELISAs (Milenia Biotec GmbH). Antimicrobial activity testing of the textiles equipped with CD-antiseptics-complexes was performed according to the JIS L 1902:2002 ('Japanese Industrial Standard' for evaluation of anti bacterial activity of textile materials) against the test organisms *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*.
Results: CHX, PHMB, and Iod exhibit a negative effect on proliferation of HaCa Tkeratinocytes *in vitro*. In contrast, the CD-antiseptics-complexes displayed improvedcell compatibility. Hence, a textile material of cotton wool without intrinsic antimicrobial properties was equipped with the β-CD-complexes of the antiseptics. Textile samples with the respective β-CD-antiseptics-complex showed a significant to strong reduction of *S. aureus* and *E. coli* growth. β-CD/PHMB-finished textiles also exhibited a strong antifungal effect and achieved a complete inhibition of yeast growth. Textile with β-CD/CHX showed a significant antifungal activity against *C. albicans* while samples with β-CD/Iod had no effect on *C. albicans*.
Discussion: The use of cyclodextrins enables a new form of loading textile materials with antiseptics such as polihexanide, chlorhexidin diacetate, and iodine for disinfecting acute wounds or for application on chronic wound care. Textiles equipped with β-CD-antiseptics-complexes possess a high antibacterial activity and samples with β-CD/PHMB und β-CD/CHX also showed antifungal properties. Furthermore, the study shows that the complexes of α-, β-, and γ-Cyclodextrinen with the antimicrobial substances exhibit a better cell compatibility *in vitro*.

P257
Health care and psychosocial situation of patients with neurofibromatosis type1 in Germany

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Introduction: Neurofibromatosis type 1 (NF1) is a rare autosomal dominant disease(incidence 1:3000) characterized by multiple benign tumors of the peripheral nerves and typical skin disfigurements. Due to complexity and phenotypic variety of this disorder, diagnosis, medical care and management of NF1 are challenging. Nevertheless, there are few NF1 specialized centres in Germany and there is a lack of studies about the patients' health care and psychosocial situation.
Methods: In a non-interventional study $n = 228$ patients filled in a questionnaire on clinical features, consulted physicians and non-medical support options, treatment benefits and other health care parameters. Subgroups of patients with versus without specialist treatment were compared.
Results: The participants had a mean age of 44 (±13), 61.7% were female. Morethan 90% of the patients reported caf au lait spots and cutaneous neurofibromas.37% of the patients stated to be diagnosed with a depressive disorder. In 43% the diagnosis of NF1 was delayed for more than 3 years. Most patients were treated by general practitioners (67.5%), NF-1 specialists (59.2%) and/or neurologists (43.2%). Patients with medical care by specialists were more satisfied with the time required for diagnosis, information about the disease and general health care for NF. Consequently, their overall patient benefit index was significantly higher. However, specialists did not considerably differ from non-specialists with regard to psychosocial support. 48.3% of the patients had relatives affected by NF-1. These patients had more long-term relationships and more had children.
Conclusions: These results indicate that specialists for NF1 provide better medical care but do not meet the patients psychosocial needs better than non-specialists.
Psychosocial support should be part of clinical care of NF1 patients. Relatives affected by the disease might help to cope with certain aspects of daily life challenges leading to a better psychosocial integration. Dermatologists are of significance since skin manifestations are the most frequent symptoms of NF1patients.

P258
How do tertiary referral centers in Germany approach chronic spontaneousurticaria?

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Background: The EAACI/GA2LEN/EDF/WAO-guidelines for urticaria recommend totreat the underlying cause of chronic spontaneous urticaria (csU) when possible. In 2009, a nation wide survey study demonstrated that many but not all dermatologists in a private practice setting know and follow the

current guidelines and try to identify an underlying cause in their csU patients. The rate of successful identification of acause was reported to be 24% on average. Since the results also showed that one out of four patients is referred to a specialized clinic or center, we analysed the diagnostic approaches and outcome of diagnostic programmes of urticaria specialists.
Methods: During a standardized expert-to-expert interview, tertiary urticaria referral centers were assessed for their knowledge of the current guidelines as well as their programmes to identify underlying causes in csU patients. In total, data of 41 specialist centers (20 from a university and 21 from a non-university hospital Department of Dermatology) were analysed.
Results: Urticaria specialist centers reported to see 25 csU patients per month on average. Ninety-five percent claimed to be familiar with the current guidelines. All centers reported to have programms for the identification of underlying causes in csU patients with average success rates of 45%3%. While all hospitals reported to perform laboratory tests such as a differential blood count and determination of BSG/CRP, all other selectable diagnostic options mentioned during the interview were chosen slightly less often (detection of thyroid hormones and anti-thyroid antibodies (98%), microbiologic examinations (95%), detection of total IgE (95%), consultation of an ENT-specialist (95%), serologicanalyses (93%), detection of autoimmune parameters (93%), pseudo allergen-low diet (90%), body imaging (88%), consultation of a dentist (85%), skin prick testing (85%), autologous serum skin test (85%), provocation tests (76%). As underlying causes of csU, the centers reported to identify most commonly: Infections (41%), drugs (20%), intolerance (17%) and auto reactivity (16%).
Conclusions: Virtually all of the participating centers attempt to identify underlying causes in csU patients by using abroad spectrum of different measures. This leads to a successful identification of underlying causes in almost half of the patients, which seems to be considerably more successful as compared to the private practice setting.

P259
Updosing of antihistamines in urticaria treatment – the patients' perspective

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Background: The first line treatment of chronic spontaneous urticaria are non-sedating H1-antihistamines. However, many patients do not respond sufficiently to approved doses. In these cases, the current guidelines recommend to updoseH1-antihistamines up to fourfold. As of yet, it is largely unclear how chronic spontaneous urticaria patients perceive treatment with H1-antihistamines in standard and higher than standard doses.
Methods: In a nationwide survey, patients with chronic spontaneous urticaria who had received H1-antihistamine treatment were asked about their experience. In total, 319 completed surveys of patients from allover Germany were available for analyses.
Results: Seventy-five percent of all participants had experience with up dosing of H1-antihistamines. Absence of efficacy of the standard dose was the most commonly reported reason for increasing the dose. Around half of the patients (51%) reported that they had concerns regarding the step of up dosing. These included fear of adverse drug effects (26%), side effects of long term use (23%), loss of efficacy over time (19%) and drug addiction (9%). In 45% of reported events of H1-antihistamine up dosing, this treatment was rated to be solely effective or clearly more effective than standard dosed H1-antihistamine therapy. While the reported frequency of adverse effects did not differ considerably between high and standard H1-antihistamine doses (34% and 33%), the magnitude of side effects (most commonly sedation) might increases during up dosing: Of 102 reported events of side effects during up dosing, 35 were rated to be clearly (38%) and 39 to be slightly more intense (34%) as compared to the side effects that had appeared during treatment with standard doses of the same drug.
Conclusions: Taken together, these data show that patients with chronic spontaneous urticaria who receive higher than regular H1-antihistamine doses commonly experience better control of symptoms and that 'side effects' are perceived to be stronger by some patients. Up dosing ofH1-antihistamines in chronic spontaneous urticaria should be preceeded by in depth patient information, addressing all relevant aspects including the adverse effects profile of the H1-antihistamine to be used.

P260
Influence of negative pressure wound therapy (NPWT) on fibroblasts in 3D-culture

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Introduction: NPWT has been shown to be clinically effective in the treatment of chronic-stagnating wounds. However, the exact mechanism of action on wound healing still remains to be elucidated. We have established an *in vitro* model for NPWT on chronic wounds using fibroblast in a 3D-culture system to investigate the influence of NPWT with different wound dressings on cell viability and migration.
Materials & Methods: Fibroblasts were seeded on collagen pellicles and cultured for14 days. The wound dressing samples (AM-gauze*, large-pored-foam**) were placed on the cultures; this assembly was positioned in a six-well plate and sealed with avacuum-applicator-lid (VAL). VALs were connected to medium supply and vacuum pump. Experiments were carried out at -80 mmHg and -120 mmHg for 48 h. Static controls were run at each assay. Histology specimens were stained with haematoxylin/eosin and anti-vimentin. Cell viability and ingrowths of cells into the wound dressing samples was determined.
Results: NPWT decreased fibroblast viability compared to static controls. No difference between cells treated with -80 and -120 mmHg was observed. The cells responded to the subatmospheric pressure by migrating in direction of the applied vacuum. Wound dressings affected cell migration differently; in cultures treated with AM-gauze* cells were localized at the pellicle edge, while cells continued to migrate into the large-pored-foam**.
Conclusions: This study suggests that the positive effects of NPWT may result from the recruitment of cells to the wound site, where they contribute to formation of granulation tissue. The dressings used for NPWT exhibit different effects. While fibroblasts did not migrate into the fine-grained AM-gauze*, they showed a significant tendency to grow into the large-pored-foam**.
*Kerlix AMD, Kendall; **V. A. C. Granu Foam Dressing, KCI.

P261
Crucial role of tumor necrosis factor-α in the pathogenesis of acute spongioticeczematous dermatitis

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Apoptosis of single keratinocytes (KC) is a characteristic feature of spongiosis formation, the histopathologic hallmark of acute eczematous dermatitis. In acute eczema, activated dermis-infiltrating T cells secrete several pro inflammatory cytokines which might be decisive for KC apoptosis or survival.
We analyzed the role of tumor necrosis factor-α (TNF-α) in the decision of KC fate during spongiosis formation in acute eczematous dermatitis. Supernatants of activated human CD4+ T cells induced apoptosis in primary KC, which could be fully inhibited by individual blockade of interferon-γ (IFN-γ) and CD95 but not by neutralization of TNF-α activity. As compared to CD95-triggering alone, synchronousCD95 and TNF receptor cross-linking in the presence of IFN-γ only marginally enhanced KC apoptosis. Importantly, pre-treatment of KC with TNF-α followed byCD95 stimulation, but not vice versa, significantly amplified KC apoptosis as compared to CD95 stimulation alone. This TNF-α mediated sensitization toCD95-induced KC cell death could be abrogated by blocking TNF receptor 1(TNF-R1) but not TNF-R2 mAb. In eczematous dermatitis CD95 was expressed throughout the epidermis, whereas immunohistological detection of TNF-R1 was rather restricted to KC around spongiotic vesicle formation.
Thus, TNF-α primes KC for CD95-mediated signals which results in an increased susceptibility to apoptosis. TNF-R1 expression and spatial action of TNF-α restricted to spongiotic vesicles both promotes CD95-induced KC apoptosis and limits the proinflammatory KC response.

P262
Growth substrates with sulfated glycosaminoglycans induce a proliferating fibroblast phenotype *in vitro*

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Dermal fibroblasts (dFb) play a crucial role in dermal wound healing. They receive signals from immune cells, keratinocytes and are influenced by the chemical and physical properties of ECM itself which has an impact on cell proliferation, differentiation and ECM metabolism.
Here we investigated the effect of selectively designed artificial ECM (aECM) on the physiology of dFb with respect to cell proliferation and ECM synthesis.
The aECM consists of naturally occurring collagen I (Coll) and chemically sulfated glycosaminoglycans (GAG). These sulfate groups might be feasible binding partners for growth factors and cytokines thus improving acceptance of implants in the recipient tissue.
Primary human dFb from breast and foreskin were cultured on coatings of Coll: GAG mixtures of 1:1 or 10:1 ratio. Coatings were formed via fibrillogenesis of tropocollagenin presence of GAGs in aqueous solution under *in vivo* like conditions. Hyaluronan (Hya) and chondroitin sulfate (CS) were chemically modified by introduction of sulfate groups to obtain degrees of substitution of 1.0 and 3.0 for Hya and 1.8 and 3.1 for CS.
The cell proliferation was quantified by BrdU assay. Matrix synthesis was assessed by Hya ELISA, Western Blot for Coll and determination of steady state mRNA levels of Coll and Hya synthase by qRT PCR.
Higher degrees of GAG-sulfation resulted in two to three fold increased proliferation of foreskin dFb and up to eight-fold increase of proliferation of breast skin dFb within 48 h in comparison to polystyrene. Varying Coll: GAG ratios of the coatings were inconsiderable for cell proliferation. Hya accumulated during 24 h in the cell cultures. Compared to controls Hya release and Hya synthase expression were reduced by increasing sulfation levels of GAGs with at least 50% reduction of synthesis on the coatings with highly sulfated GAG derivatives.
Coll I(α1) mRNA was transiently downregulated in dFb grown on derivates Hya 3.0 and CS3.1 at 10:1 ratio for 8 h. Coatings with highly sulfated Hya and CS at a Coll: GAG ratio of 1:1 resulted in a strong down regulation of coll (1) mRNA expression after 8 and 24 h. Downregulation of collagen synthesis was verified on protein level by Western Blot for Hya3.0.
These results indicate that the sulfation of GAGs has a positive impact on cell proliferation of dFb from various tissue sources and would probably lead to fast and effective colonization of implants covered with such a ECM. The data suggest that sulfated a ECM induce a highly proliferating, non-synthesizing phenotype of dFb resembling early stages of wound healing.

P263
[Textus] balance® and [Textus] bioactiv® – antimicrobial quality and cell compatibility of functional wound dressings

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Introduction: [Textus] balance® and [Textus] bioactiv® are functional wounddressings, which consist of aqua-fibre (synthetic fibers PE/PET) and super absorbent poly acrylate. [Textus] bioactiv® further contains silver-zeolite as an active antimicrobial agent. Chronic wounds are often colonized with micro organisms or show signs of infection. Hence, we have analyzed the antifungal and antibacterial properties of the two wound dressings against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli* as well as *Candida albicans*. Furthermore, increased levels of reactive oxygen and nitrogen species (ROS, RNS) were found in chronic wounds. Antioxidative properties of functional wound dressings could improve the healing outcome. Therefore, the antioxidative effects of [Textus] balance® and bioactiv® were tested. In addition, their cell compatibility was assessed using the *in vitro* HaCaT-keratinocyte model.
Materials & Methods: According to the JIS (Japanese Industrial Standard) L1902:2002 wound dressing samples of 400 mg were analyzed. The samples were incubated with the microorganisms for 24 h at 37°C under aerobic conditions. The antioxidative effects of [Textus] balance® and [Textus] bioactiv® were measured in a chemoluminescent assay (ABEL® Antioxidant Test kit containing Pholasin® specific for superoxide and peroxynitrite, Knight Scientific Limited). Proliferation of HaCaT-keratinocytes was determined after incubation of the cells with extracts from the wound dressings with the luminometric ATP-assay ATPlite(TM) M (Perkin Elmer). For both assays, light generation was monitored using a microplate luminometer(LUMIstar Galaxy, BMG LABTECH Ltd.).
Results: Both wound dressings strongly inhibited the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Escherichia coli*. [Textus] bioactiv® was more effective against *Klebsiella pneumoniae* than [Textus] balance®, while both significantly reduced the growth of *Candida albicans in vitro*. Furthermore, [Textus] balance® and bioactiv® were able to reduce the formation of ROS and RNS in the test system. Moreover, [Textus] balance® and bioactiv® showed a high cell compatibility. Incubation of HaCaT keratinocytes with the wound dressing extracts for 24 h had no cytotoxic effect on the cells *in vitro.*
Discussion: [Textus] balance® and [Textus] bioactiv® exhibit a distinct antibacterial and antifungal activity as well as a high cell compatibility *in vitro*. In addition, they possess antioxidative properties. It is believed, that the overproduction of reactive nitrogen and oxygen species during wound healing results in an elongated inflammatory phase and severe tissue damage. The reduction of these active species as well as the removal of microorganisms seems to be a suitable way to promote wound closure. Hence, these wound dressings have the potential to improve wound care of patients with chronic wounds.

P264
A proteomic approach identifies SOD2 overexpression to be responsible for imbalanced redox signalling in senescent fibroblasts and skin ageing

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The free radical theory of ageing postulating increased concentrations of reactive oxygen species (ROS) to drive ageing is still controversially discussed. We here addressed the question whether alterations in the redox balance may modulate signaling pathways causally involved in fibroblasts senescence and skin ageing. A proteomic approach with 2D fluorescence difference gel electrophoresis and mass spectrometry identified manganese superoxide dismutase (SOD2) to be 13-fold increased in senescent fibroblasts at a cumulative population doubling (CPD) >70 compared to young fibroblasts (CPD < 22), while hydrogen peroxide $(H_2O_2)^-$ detoxifying enzymes (peroxiredoxin 1, 2, 6, glutathione peroxidase and catalase) showed only minor changes. The resulting imbalance in H_2O_2 generationmay lead to severe disruption of redox homeostasis. Increased SOD2 expression and activity were confirmed using immunostaining/blot and activity assays *in vitro* and, interestingly, also in the skin of old individuals compared to young individuals. Intracellular H_2O_2 concentrations were found to be increased in senescent fibroblasts following adenoviral transduction of the highly H_2O_2 specific biosensor HyPer. Using *in situ* techniques like DHE staining in the presence and absence of distinct ROS scavengers on skin cryosections, H2O2 was found to be increased also in skin sections from old individuals (≥65 years, $n = 5$) compared to young individuals(≤35 years, $n = 5$). H_2O_2 was identified to be responsible for the increase of totalMMP-1 and active MMP-1 in supernatants from senescent fibroblasts to further study the underlying signaling pathways, we next investigated the activation profile of the AP1 (activating protein 1) transcription factor responsible for transactivation of the TRE element in the MMP-1 promoter *in vitro* and *in vivo*. Interestingly, using lenti viral transduction of a reporter gene construct with luciferase under the control of the TRE element of the MMP-1 promoter, activation of AP1 was detected in senescent and H_2O_2-treated young fibroblasts. These data were confirmed using a specific Transcription Factor ELISA for phosphorylated cJUN representing the major constituent for active AP-1 in old fibroblasts and also by Western Blot analysis using an antibody against phosphorylated cJUN. Notably, downregulation of cJUN by specific siRNAs in senescent fibroblasts resulted in decreased MMP-1 activity, indicating that AP-1 increased transactivation in senescent fibroblasts is cJUN-dependent. Preliminary results from microarray analysis of TRE-dependent genes from senescent fibroblasts revealed that some but not all TRE-dependent genes are upregulated further supporting the specificity for the H_2O_2-dependenttransactivation of the TRE site in the MMP-1 promoter. In conclusion, we have identified SOD2 overexpression with imbalanced increase in H_2O_2 in senescent fibroblasts which – via enhanced c-JUN phosphorylation – activates AP1 and induces target genes including MMP1 eventually leading to skin ageing.

P265
Antimicrobial effect of zinc pyrithione on fungal and bacterial pathogens and anti proliferative influence on HaCaT-keratinocytes *in vitro*

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Introduction: Zinc pyrithione is widely used as an antimicrobial active agent in topical applications. It has a therapeutic value for patients with pityriasis versicolor, dandruff or seborrhoeic dermatitis. These diseases have been linked to colonization and infection with yeasts, most likely Malassezia spp. Hence, the study presented has analyzed the antifungal and antibacterial properties of zinc pyrithione *in vitro* according to the JIS (Japanese Industrial Standard) L 1902:2002. Influence of zinc pyrithione on the growth of pathogenic yeasts was further determined by micro plate laser nephelometry. In addition, the cell compatibility of zinc pyrithione was assesse dusing the *in vitro* HaCaT-keratinocyte model.

Materials & methods: According to the JIS L 1902:2002 0.2 mL samples with different concentrations of zinc pyrithione were applied on polyester for testing. The samples were incubated with bacteria (*Klebsiella pneumoniae*, *Staphylococcuss aureus*) and yeasts (*Malassezia furfur*, *Malassezia pachydermatis*) for 24 h at 37°C under aerobic conditions. Growth curves of the yeasts were further monitored using a micro plate laser nephelometer (NEPHELOstar Galaxy, BMG LABTECH Ltd.). Proliferation of HaCaT-keratinocytes was determined after incubation of the cells with zinc pyrithione dilutions up to 48 h using a luminometric ATP-assay (ATPlite(TM) M kit, PerkinElmer). The ATP dependent light generation was measured with a micro plate luminometer (LUMIstar Galaxy, BMG LABTECH Ltd.).

Results: Zinc pyrithione in concentrations >16 M significantly inhibited the growth of *Klebsiella pneumoniae* and *Staphylococcuss aureus* according to the JIS L1902:2002. It could also be shown, that a concentration of 50 M zinc pyrithione has a significant antifungal activity against Malassezia furfur and Malasseziapachy dermatis. Growth curves, which were monitored by microplate laser nephelometry, were used to determine the half maximal inhibitory concentration of zinc pyrithione for *M. pachydermatis* (0.230.01 M) and *M. furfur* (6129 M). HaCaT-keratinocytes were sensitive to low concentrations of zinc pyrithione in the long term incubation assay. A short incubation of 15 min with zinc pyrithioneexhibited less negative effects on HaCaT keratinocyte proliferation.

Discussion: Zinc pyrithione possesses distinct antibacterial and antifungal activity. In particular, its antifungal effect on Malassezia spp. demonstrates the possible therapeutic importance of zinc pyrithione for pityriasis versicolor, dandruff or seborrhoeic dermatitis. It could be shown that a subsequent washout after application can improve the cell compatibility *in vitro*.

P266
Expression profiling of the perilipin family in human SZ95 sebocytes

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The perilipin family of proteins share a highly conserved sequence organization and has been identified in diverse eukaryotic species. Their members, perilipin 1–5(PLIN 1–5) localize to the surfaces of intracellular lipid droplets of adipocytes and many other cell types. Although the presence of PLIN 1 and PLIN 2 (adipophilin) hasbeen previously demonstrated in sebaceous glands, the expression of this family has not been studied systematically in single sebocytes before.

We employed the immortalized human sebaceous gland cell line SZ95 to study the expression of the perilipin family in undifferentiated cells and at different time points(6, 12 h, 1, 2, 3, and 4 days) after induction of normal differentiation(sebaceous lipogenesis) by addition of the essential fatty acid linoleic acid and the peroxisome proliferator-activated receptor ligand ciglitazone to the culture medium. Increased differentiation during time was confirmed by staining cells with oil red and the expression of the perilipin proteins was evaluated by Western blot analysis using antibodies from Progen (Heidelberg, Germany). Expression of PLIN 5 (OXPAT, MLDP) and PLIN 4 (S3-12) was not detected at any differentiation stage. PLIN 3(TIP47) was expressed at every stage analyzed with a slight increase after day 2. PLIN 2 was also expressed at every stage but the increase was more dramatic and clearly observable already 12 h after the start of the differentiation. The employed antibody failed to detect expression of PLIN 1 in the samples by Western blots, although it stained lipid droplets in sebaceous glands following immuno histochemistry. Our study will be completed by Northern blot and immunohistochemical analysis.

In conclusion, our findings reveal that the basal and sebaceous lipogenesis-induced expression of the perilipin family in cultured sebocytes differ considerably from the pattern observed in other cell types. This may reflect the distinct properties of sebaceous lipogenesis as compared to lipid production in adipocytes.

P267
Macrophages as sentinels directing the quality of skin repair

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Substantial evidence in different model organisms indicates that the immune systemis of primary importance in determining the quality of the repair response, including the extent of scarring as well as the restoration of organ structure and function. However, the relationship between repair and the immune response is complex and not completely understood. Indeed, there is evidence for both negative and positive roles. To unravel the dual role of the innate immune response in diverse repair mechanisms, we developed a novel mouse model that allows conditional depletion of macrophages during the sequential stages of the wound healing response. Depletion of macrophages restricted to the early stage of the repair response (inflammatory phase) significantly reduced the formation of a vascularized granulation tissue and impaired epithelization as well as wound closure kinetics. Furthermore, differentiation of macrophages which entered the wound site at later stages, towards anM2-phenotype was attenuated. However, these wounds revealed minimal scar formation. In contrast, depletion of macrophages restricted to the consecutive mid stage of the repair response (phase of tissue formation) resulted in severe hemorrhage in the wound tissue. Under these conditions, transition into the subsequent phase of tissue maturation and wound closure did not occur. Finally, macrophage depletion restricted to the late stage of repair (phase of tissue maturation), after epithelization was complete, did not significantly impact the outcome of the wound healing response. These results demonstrate for the first time that macrophages exert distinct functions during the diverse phases of skin repair, which are critical to control the natural sequence of repair events.

P268
Novel findings on SERPINs as critical regulator of skin repair

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Serin Protease Inhibitors (SERPINs) have evolved to be the predominant plasma proteinase inhibitors which inactivate serine proteinases, particularly also leukocyte-derived proteases. Yet, understanding their specific role in skin injury and repair remains a challenge. In this study we investigated the function of α1-antichymotrypsin (α1-ACT, SERPIN A3) during normal and impaired wound healing conditions in mouse and human. Following skin injury gene expression of α1-ACT, and its mouse homologue Spi-2, was strongly induced and exhibited the kinetic of a classic acute phase response in healthy humans and wild type mice, respectively. As revealed by immunohistochemical staining keratinocytes and infiltrating leukocytes contribute to α1-ACT activity at the wound site. This finding was unexpected because up to date the liver has been shown to be the major source forα1-ACT. In addition, Spi-2 expression following skin injury in healing impaired diabetic mice was almost absent, suspecting a role in tissue repair. Indeed, topical application of recombinant α1-ACT (rα1-ACT) into wounds rescued the diabetic impaired healing phenotype, corroborating its critical function during the wound healing response in skin. rα1-ACT is likely to promote the healing response by reducing tissue damage mediated by potent leukocyte-derived enzymes, includingcathepsin G, that are released at the proinflammatory diabetic wound site. To investigate the clinical relevance of our findings, we quantified α1-ACT activity in healing and non-healing human wounds. The majority of exudates derived from non-healing wounds revealed a significantly decreased activity of endogenousα1-ACT when compared to that of normal healing human wounds. Interestingly, the level of endogenous α1-ACT activity in wound exudates correlated inversely with the exudates EUR(TM) potency to degrade and subsequently inactivate rα1-ACT (rα1-ACT). LC-MS analysis of rα1-ACT cleavage fragments identified new cleavage sites within the Reactive Center Loop and together with protease inhibitor studies showed that neutrophil elastase was the predominant protease involved in uncommon α1-ACT degradation and inactivation at the chronic wound site. Collectively, these results reveal novel functions for local α1-ACT in the acute phase response following skin injury; provide mechanistic insights into its function during the repair response and raise novel perspectives for its potential therapeutic value in impaired wound healing states.

P269
Cockayne syndrome revised: DNA-repair failure orribosomopathy?

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Cockayne syndrome(CS) is a devastating childhood disease characterized by premature aging traits as neurological degeneration, cataracts and cachexia followed by early infant death. It is a polygenic disease- the recessive mutation of five different genes can cause Cockayne syndrome. The gene products are all involved in nucleotide excision repair of UV-lesions so CS is commonly attributed as a DNA-repair disease. A total failure of nucleotide excision repair is typical for XPA patients, followed by Xeroderma pigmentosum, a cancer-prone skin disease without childhood degeneration and infant death. Thus alternative redundant functions of the causal genes are investigated. Here we present evidence that all five genes involved in the pathogenesis of Cockayne syndrome are part of ribosomal biogenesis i. e. RNA polymerase I transcription. All gene products have been identified to bind to the rDNA promoter *in vivo* and gene internal regions. Three genes (CSB, XPB, XPD) are involved in transcription elongation of RNA polymerase I, the other two are currently under investigation. As disturbances in ribosomal biogenesis ('ribosomal stress') are followed by p53 mediated cell cycle arrest and apoptosis, premature aging of CS patients might be due to a dysbalance between ribosomal biogenesis and p53 activation.

P270
Einfluss von Fumarsäureestern auf die NF-E2 Related Factor 2 (Nrf2) abhängige mRNA expression von hämoxygenase-1 (OH-1) und NADPH-chinon-oxidoreduktase (NQO1) in primären humanenKeratinozyten

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Fumarsäureester (FAE) sind in der systemischen Therapie der mittelschweren bisschweren Psoriasis etabliert. Der Wirkmechanismus ist bis heute noch nichtvollständig geklärt. Insbesondere die Auswirkung der Modulation von intrazellulăremGlutathion (GSH) durch Fumarsäuredimethlyester (DMF) scheint für das Wirkprofilessentiell zu sein. An eine Veränderung von intrazellulären GSH-Spiegeln sindwiederum unterschiedlichste zelluläre Effekte geknüpft. So werden antioxidativeSysteme nach einer GSH-Depletion verstärkt exprimiert. Der negative Einfluß vonreaktiven Sauerstoffspezies (ROS) in der Pathogenese der Psoriasis wurdeausführlich beschrieben. Hervorzuheben ist der Einfluss von ROS auf Proliferationund Differenzierung von Keratinozyten, die im Rahmen einer psoriatischenEntzündung eine zentrale Rolle einnehmen.

Aus diesen Zusammenhängen ergab sich die Hypothese, dass DMF seineantipsoriatischen Effekte teilweise über die Regulation von intrazellulärem GSH inKeratinozyten und die damit verbunden Induktion von antioxidativen Mechanismenvermittelt.
Zur Prüfung dieser Hypothese wurde der Einfluss von DMF und seinemHydrolyseprodukt Fumarsäuremonomethylester (MMF) auf die Nrf-2 abhängigeExpression von HO-1 und NQO-1 in primären humanen Keratinozyten untersucht. Hierzu wurden die Zellen mit 10 M DMF oder MMF für 6 h inkubiert und dieentsprechende mRNA mittels RT-PCR bestimmt. Um die zellulären GSH-Spiegel vor-Inkubation mit den FAE zu erhöhen, wurden die Zellen in weitern Versuchen mit 1 mM N-Acetylcystein (NAC) für 1 h vorbehandelt und anschließend mit FAE fürweitere 6 h inkubiert.
Nach Inkubation mit DMF für 6 h war ein signifikanter Anstieg der HO-1 und derNQO-1 Expression zu beobachten. Mit MMF ließ sich eine deutliche Zunahme derNQO-1 mRNA feststellen. Allerdings blieb die Nrf-2 mRNA Expression unverändert. Die durch FAE vermittelten Effekte ließen sich nicht durch die Vorinkubation mit NACfür 1 h neutralisieren.
Zusammenfassend lässt sich feststellen, dass bei Verwendung von Keratinozyteneine Modulation von Nrf2 nicht gezeigt werden konnte, jedoch ein Einfluss aufnachgeschaltete GSH-abhängige Signalkaskaden wie HO-1 und NQO-1 vorhandenwar. In weiteren Versuchen sollen die beschriebenen Effekte auch auf Proteinebenenachvollzogen werden.

P271
Increased EGFR activity induces epidermal thickening and retards the initiation of hair follicle cycling in Dsk5 mice

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The epidermal growth factor receptor (EGFR) plays an important role in the homeostasis of the epidermis and hair follicle (HF). Accordingly, its de regulation rapidly results in disorders as inflammatory responses, tumorigenesis, and impaired wound healing. Mice completely lacking EGFR die during embryonic development ora few weeks after birth, depending on the genetic background. Surviving EGFR-deficient mice and mice carrying hypomorphic mutations of the receptor develop a delayed and fuzzy coat, showing a severe phenotype of aberrant and premature hair follicle differentiation, epidermal atrophy, and low epidermal keratinocyte proliferation rates. Dsk5 (dark skin 5) mice have been generated by chemical mutagenesis and bear a point mutation (Leu863Gln) in the kinase domain of the EGFR resulting in increased tyrosine kinase activity. Dsk5 mice were detected because of their excessive footpad pigmentation. Here, we analyzed epidermal development and hair follicle morphogenesis and cycle induction during early postnatal life in Dsk5 mice as compared to their control littermates. Histological examination of defined regions of back skin revealed no differences in hair follicle morphogenesis between Dsk5 mice and control littermates at postnatal day 8.5. However, the thickness of both the epidermis and the dermis of Dsk5 mice was significantly increased at this stage as compared to control mice (with remarkable thickening of the stratum corneum and granulosum). At postnatal day 19.5, Dsk5 mice showed a significant retardation in the initiation of hair follicle cycling. Additional stages of HF developmental and cycling as well as proliferation and survival of Dsk5HF keratinocytes are being currently evaluated. The alterations observed in the skin of Dsk5 mice confirm the importance of this system for epidermal and HF biology. The rather mild phenotype observed supports the concept that operating negative feedback regulatory mechanisms counterbalance the increase in EGFR activity in this mouse line.

P272
Outer root sheath melanocytes, arts and grafts

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Vitiligo is a local skin depigmentation disorder, due to the lack of melanocytes in epidermis or their function. Vitiligo appears in 0.5 % of the Northwest European population and as high as 8% at the regions where dark skin prevails. The symptoms are usually fully developed by the age of 20. The white patches, even though physiologically benign, bring about serious psychological disturbance and tremendously impact life quality.
Conservative therapies for Vitiligo remain palliative and short-term. Lately, causative therapies are being developed, addressing melanocyte absence or in capacitance by transplanting them to the de pigmented patches. Non-invasive, low-sample biopsiesare a definitive 'one-up' when it comes to the cell therapy of Vitiligo. The Outer Root Sheath (ORS) technology offers a painless and completely harmless biopsy of the ana gene hair and development of epithelial cells from the resident adult stem cell pool of the hair root.
Euroderm Biotech & Aesthetics, pioneers in the field of ORS-derived skin products, have successfully launched a keratinocyte-based skin transplant, Epidex®, cultivated from a small sample of autologous hair roots, into clinical trials and onto the market. The product was designed for chronic wound treatment. By embedding ORS-derived melanocytes into an already functional skin transplant, our Group at Translational Centre for Regenerative Medicine is working hand-in-hand with the Department of Dermatology, Venerology and Allergology, University Clinic in Leipzig towards a promising autologous ORS-derived, transplantation-based Advanced Therapy Medicinal Product, designed for the treatment of Vitiligo.
Each graft development involves a choice of a suitable carrier. New generation of tissue0-compatible carriers serve not only as biodegradable mechanical support, but as a non-toxic delivery system as well. Main candidates for such biocompatible devices are Collagen Type I extracellular Matrix (EXM) and Poly caprolactone scaffold (PCL). Collagen as the most abundant protein of the skin offers high compatibility as a graft carrier. Poly caprolactone is a neutral, non-toxic biodegradable polimer. It is also able to retain active substances in the course of preparation and gradually release the retained material, hereby also serving as a delivery system. Melanocytesare capable of populating such mesh and adhering to the fiber. moreover, they assume all the features of ripe melanocytes – correct melanocyte star-like morphology, expression of Tyrosinase and glycoprotein 100 melanocyte markers, active synthesis of melanin and preparation for its delivery.
This study deals with the ORS-derivation of melanocytes, biocompatibility ofmelanocytes and graft carriers such as EXM and PCL and their therapeutic potential.
All of the above speaks in favour of EXM and PCL as friendly and biocompatible carriers for epidermal equivalent grafts.

P273
Epidermal calcium concentrations in murine atopic dermatitis visualized by fluorescence lifetime imaging

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Calcium is a major player of regulation of keratinocyte differentiation and proliferation. It is involved in establishing barrier function of skin which is, in part, maintained through Ion-selective tight junctions localized in the Stratum granulosum (SG), the cornified envelope in the Stratum corneum (SC), and the extracellular lipid matrix of the SC. Earlier experiments showed a calcium gradient in normal skin increasing from the basal layer to its peak in the Stratum granulosum and an abrupt drop in the lower Stratum corneum.
Atopic dermatitis is associated with an impaired epidermal barrier function, increased epidermal proliferation and changes in differentiation of epidermal keratinocytes. Several reports indicate changes of epidermal calcium and an influence of external calcium on eczema.
We here are using a defined inducible murine atopic dermatitis model, OVA, to assess changes of the epidermal calcium distribution compared to normal skin by using two-photon fluorescence lifetime imaging microscopy (FLIM).
We reproducibly induce an eczema as evidenced in gross morphology (erythema and scaling), a broadened epidermis in standard histology (H&E), and function (increased TEWL).
Our first results in FLIM-experiments show increased calcium values throughout a major part of the broadened epidermis with a less steep increase, where normal, untreated murine skin shows a narrow epidermis with lower calcium values overall, increasing towards the SG.
Results on changes in the epidermal calcium distribution dependent on the state of the eczema will be presented.

P274
Knockdown of the novel retinol transporter STRA6 leads to hyper proliferation of keratinocytes in 3D human skin equivalents

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Retinoids play a key role in cell proliferation and differentiation. Retinol cannot be synthesized de novo and is bound to retinol-binding protein (RBP) during its transporting the blood circulation from liver to target tissues. Recently STRA6, a multi transmembrane domain protein, was identified as a high affinity specific membrane receptor for retinol-RBP in bovine retinal epithelium cells. STRA6 removes retinol from RBP and transports it across the plasma membrane into the cytoplasm. To determine whether similar transport processes take place in human skin cells, we analyzed expression of human STRA6 in normal human epidermal keratinocytes (NHEK), a keratinocyte cell line (HaCaT) and human dermal fibroblasts. Quantitative RT-PCR analysis detected a constitutive expression of STRA6 in all investigated skin cells. STRA6 expression is significantly up regulated by ligands of various nuclear retinoic acid receptors (RAR) such as 9-cis-RA, 13-cis-RA, all-trans-RA and targretin as well as retinol itself. In contrast STRA6 expression was unaffected by exposure to ligands of other class II nuclear receptors including phenobarbital, dexamethasone and benz(a)anthracene. Furthermore to investigate the influence of STRA6 knockdown on the physiological function and morphological structure of human skin we established a human 3D skin model with STRA6 stable knockdown cells. To further investigate the influence of STRA6 on epidermal proliferation we generated HaCaT cells with stable knockdown (up to 96%) of STRA6 using shRNAs from lentiviral vectors. We then established a human 3Dskin model using the STRA6 stable knockdown HaCaT cells. Cells transduced with lentivirus non-target vector served as controls. Within 7 days the epidermis in the STRA6 knock down 3D model showed increased proliferation as compared to the control. This was confirmed by immunohistochemical staining for keratin 16 (K16), which served as a marker for hyper proliferation. By 14 days the knock down 3D model displayed a three-fold thicker epidermis than did the control. In conclusion our results indicate for the first time that STRA6 is constitutively expressed in human skin cells and that it is essential for the active uptake of RBP-bound retinol into these cells. This facilitated uptake may play an important role in retinoid signaling in human skin.

P275
Effect of cytokines on transglutaminase 1 expression in human keratinocytes

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The disturbed skin barrier plays an important role in the pathogenesis of atopic dermatitis (AD). The reason for the disturbed skin barrier function in AD is only partly known. Besides loss of function mutations in the filaggrin gene in about 30% of the patients there are data suggesting a secondary impairment of the skin barrier by mediators of inflammation. In line with this we found decreased filaggrin expression in skin from patients with or without filaggrin mutations. In addition, we previouslydescribed a disturbed expression of the cornified envelope proteins involucrin and loricrin in AD. Transglutaminase 1 plays an important role in the cross-linking of cornified envelope proteins and the covalent attachment of lipids to involucrin during skin barrier formation. We investigated the influence of the proinflammatory cytokinesIL1 beta, IL4, IL5, IL6, IL13, IL15, IL17, IL18, IL28, IL31, IFN gamma, TNF alpha und TGF alpha on the expression of transglutaminase 1 in human keratinocyte cultures. Analysis was semiquantitatively performed by real-time PCR. None of the cytokines investigated caused a decrease of transglutaminase 1 expression. However, IFN gamma caused a significant increase of transglutaminase 1 expression; it has been described that this stimulation also occurs in psoriasis *in vivo*. Stimulation of transglutaminase 1 by INF gamma may prevent a significant disturbation of the skin barrier in psoriasis. In AD the lack of the TH1 cytokine INF could aggravate barrier dysfunction.

P276
Functional importance and hormonal regulation of nestin expression in multi potent adult human skin progenitor cells *in situ* and *in vitro*

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Neurofilament nestin+ cells of adult human skin are one focus of cell-based regenerative medicine strategies, since they may serve as an easily accessible source of adult, autologous progenitor cells for the generation of different tissue lineages. While the cultivation of nestin+ progenitors from murine and human skin have been achieved by several laboratories, it remains unclear which specific functional role(s) the expression of nestin itself plays in these progenitors, e.g. in terms of cell growth, differentiation, cell fate and hormonal regulation. To address these questions, we organ-cultured nestin+ progenitor-rich human scalp skin and isolated sweat gland (SwG)-derived nestin+ cells in the presence or absence of the adipokin leptin, which we had previously shown to up-regulate intra-mesenchymal nestin expression in human skin *in situ*. Therefore, leptin was used as a tool to up-regulate nestin expression. In addition, nestin expression was knocked-down by RNAi in cultured SwG-derived nestin+ cells. Both experimental approaches revealed that strong nestin expression is associated with increased cell proliferation and nestin expression is essential for the glial cell, but not for the neuronal cell fate of nestin+ cells. After leptin stimulation, nestin+ progenitors managed to continue on a glial differentiation path *in vitro* even after (likely incomplete) nestin silencing. As nestin+ cells could be differentiated into glial and neuronal derivatives, we conclude that nestin expression alone is primarily responsible for the glial differentiation fate of nestin+ progenitors and that leptin administration may facilitate the isolation, extended culture, and glial differentiation of primary, adult human skin-derived nestin+ stem cells.

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Interleukin-1β interferes with the balance between proliferation and differentiation through insulin resistance in human keratinocytes Vimplications for psoriasis pathogenesis

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Interleukin-1β (IL-1β) not only plays a crucial role in the pathogenesis of psoriasis, but also induces insulin resistance in metabolic tissues. Therefore we asked whetherIL-1β can confer similar effects that might contribute to the manifestation on the disease in the skin.

Using HaCaT cells we found that IL-1β indeed renders keratinocytes resistant to insulin dependant activation of the PI3-K/PKB cascade, which is mediated by p38 MAPK. We also found that insulin induces cell proliferation and expression of cytokeratin 10, a marker of terminal differentiation, suggesting that insulin drives differentiation of healthy keratinocytes. This effect is blunted under chronic IL-1β treatment, resembling the inflammatory situation in psoriasis, where keratinocytes differentiation is abnormal and shifted towards hyperproliferation.

Surprisingly we found that in the psoriatic plaque PKB/Akt is hyper activated. This effect seems to be mediated by IL-1β, which *in vitro* can transiently activate the PKB cascade. This effect is not only mediated via PI3-K but also via p38 MAPK, IKK andJNK. This leads to IL-1β dependent proliferation of keratinocytes.

We provide evidence that under healthy conditions insulin regulates the equilibrium between differentiation and proliferation of keratinocytes which is the prerequisite for proper formation of the epidermal layers. Under conditions of systemic inflammation such as psoriasis, high levels of IL-1β in the skin lead to blockade of differentiation by means of insulin resistance. At the same time IL-1β promotes hyper proliferation of keratinocytes. Both mechanisms contribute to the formation of the psoriatic plaque. Thus, controlling correct insulin signaling in the skin might represent a novelanti-psoriatic strategy.

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Comprehensive analysis of activated signalling components in psoriatic skin lesions

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Psoriasis is a chronic inflammatory skin disease with a prevalence of 2–3% in the population, characterized by red scaly plaques which may occur on any site of the body. Although biologics directed against different cytokines, e.g. TNF-α, show promising results in the therapy of the disease, a comprehensive analysis of dysregulated signalling components that might represent novel therapeutic targets is still missing.

Therefore, we investigated the expression, activation and distribution of signalling components of the PI3-K, mTOR pathway and several MAPK cascades such as JNK/SAPK, p38 MAPK, ERK1/2. Crysections of lesional or unaffected skin of the same patients were stained using immunohistology with a panel of phospho-specific antibodies. In addition lysates of psoriatic or non-lesional skin were prepared and subjected to Proteome Profiler Antibody Arrays (R & D Systems), which allowed quantification of the activation status of 46 different kinases.

We could show hyper activation of kinases known to be playing a role in psoriasis as well as novel kinases that play a prominent role in cell proliferation and differentiation. For example activation of members of the STAT family or the MAPK family could be measured. Interestingly activation of the mTOR signalling cascade in psoriatic skin was a novel finding. The mTOR complex is an important integrator of inflammatory and proliferative stimuli; therefore our data represent an interesting start point for studying the role of this complex in the pathophysiology of psoriasis.

In summary, results from this approach may point towards novel targets for the development of anti-inflammatory therapies.

P279 (V29)

Matriptase-1 expression is lost in psoriatic skin lesions and is regulated by TNF-alpha in primary human keratinocytes

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Background: The type II serine protease matriptase-1 has been shown to be indispensable for regular terminal differentiation of epidermal keratinocytes.

Objectives: To investigate the expression and regulation of matriptase-1 in primary human keratinocytes (KC) and in psoriatic skin.

Methods: Matriptase-1 expression in KC was examined by Western blot analysis and immunofluorescence staining of psoriatic and adjacent normal skin. Matriptase-1 activity was determined in epidermal lysates using Boc-Gln-Ala-Arg-AMC as substrate. To investigate the regulation of matriptase-1 *in vitro*, monolayer cultures and organotypic skin cultures of human primary KC were treated with TNF and analyzed by RT-PCR, Western blotting and immunofluorescence staining. An involvement of the nuclear factor kappa B (NFκB) signaling pathway was investigated by adenoviral over-expression of a dominant negative form of IKK2.

Results: Matriptase-1 expression was detected in the stratum granulosum of organotypic skin cultures *in vitro* and human skin *in vivo*. Western blot analysis, immunofluorescence staining and activity assays revealed that both matriptase-1 expression and enzymatic activity was strongly reduced in psoriatic skin lesions as compared to the uninvolved adjacent skin of the same donors. Exposure of KC to TNF led to a strong down-regulation of matriptase-1 mRNA and protein production *in vitro*. Organotypic skin cultures treated with TNF showed an enhanced and disturbed KC-differentiation, accompanied by complete loss of matriptase-1 expression. Inhibition of the IKK2/NFκB signaling pathway completely blocked TNF induced down-regulation of matriptase-1.

Conclusion: Since matriptase-1 is involved in regular terminal KC differentiation, its absence in psoriatic skin lesions might contribute to the barrier alterations in this disease. Blocking the IKK2/NFκB-pathway might represent an interesting target forthe treatment of psoriasis.

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Human and mouse collagen XVII show differences in shedding behavior

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The epithelial adhesion molecule collagen XVII represents a transmembrane component of hemidesmosomes. In our previous studies we have extensively analyzed ectodomain shedding of human collagen XVII within its extracellular linker domain NC16A. This is catalyzed by metalloproteinases of the ADAMs family and depends rather on structural molecule motifs than on specific amino acid sequences. Since the extracellular linker domain of human and murine collagen XVII shows high amino acid diversity, the goal of this study was to compare shedding of transiently transfected human and mouse collagen XVII constructs with defined deletions within their linker domain. In previous studies we have already analyzed ten human collagen XVII deletion constructs. Here, we investigated six linker domain deletion constructs of murine collagen XVII. Their normal membrane integration and Golgi transition was demonstrated by cell surface biotinylation and Endo H in sensibility. The shortest deletion of human collagen XVII which resulted in non-shedding was a 20 amino acid deletion spanning Ale528 to Glu547. In contrast, deletion of 20 corresponding amino acids in murine collagen XVII (Glu534 to Glu553) do not resultin decreased shedding. It revealed that the shortest deletion in murine collagen XVII which led to complete loss of shedding was a 32 amino acid deletion, from Lys513 toSer544. Secondary protein structure predictions of human and mouse linker domains indicated significant differences in their molecular structures. Our results suggest that human and murine collagen XVII molecules vary in their extracellular linker.

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DNase 2 degrades DNA on the skin surface

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The stratum corneum is an efficient barrier to the passage of genetic material, i.e. nucleic acids. It contains enzymes that degrade RNA and DNA which originate from either the living part of the epidermis or from infectious agents of the environment. However, the molecular identities of these nucleases are only incompletely known at present. Here we performed biochemical and genetic experiments to determine the main DNase activity of the stratum corneum. DNA degradation assays and zymographic analyses identified the acid endonucleases L-DNase II, which is derived from serpinB1, and DNase 2 as candidate DNases of the cornified layer of the epidermis. siRNA-mediated knockdown of serpinB1 in human *in vitro* skin models and the investigation of mice deficient in serpinB1a demonstrated thatserpinB1-derived L-DNase II is dispensable for epidermal DNase activity. By contrast, knockdown of DNase 2 reduced DNase activity in human *in vitro* skin models. Moreover, the genetic ablation of DNase 2 in the mouse was associated with the lack of acid DNase activity in the stratum corneum *in vivo*. The degradation of endogenous DNA in the course of cornification of keratinocytes was not impaired by the absence of DNase 2. Taken together, these data identify DNase 2 as the predominant DNase on the mammalian skin surface and indicate that its activity is primarily targeted to exogenous DNA.

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Overexpression of S33Y-beta-catenin in primary melanocytes and in melanoma cells abrogates mitotic spindle formation and induces cell death

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The protein beta-catenin has three different tasks in a cell. As a key element of theWnt-signaling pathway it regulates the transcription of various genes, such as C-JUN, Cyclin D1 and MITF. At the cell membrane, it links cadherins with the cytoskeleton at the adherens junctions. It is also known that beta-catenin plays a role during chromosome segregation and centrosome separation. Beta-catenin is over expressed in different types of cancer, including melanoma. We were able to 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 investigate the role of beta-catenin in melanoma formation and progression, we overexpressed a stabilized mutant of beta-catenin (S33Y-beta-catenin) in primary melanocytes and radial growth phase melanoma cells by adenoviral gene transfer. Overexpression of S33Y-beta-cateninresulted in activation of TCF/LEF/beta-catenin mediated transcription of target genes, loss of adhesion to the culture plate and cell cycle arrest. Interestingly, the cells survived in a detached state for 3 days, developed a polyploidy DNA-content and died subsequently. Microarray analysis of primary melanocytes expressing S33Y-beta-catenin revealed changes in expression of genes involved in cell adhesion, differentiation and chromosome segregation, as well as genes of the p53 pathway. Immunohistochemical analysis revealed that primary melanocytes and radial growth phase melanoma cells overexpressing beta-catenin are not able to forma proper mitotic spindle which prevents a separation of the chromosomes during mitosis. Interestingly, a part of the S33Y-beta-catenin expressing melanocytes and melanoma cells was able to skip the mitotic spindle assembly checkpoint, progressed to G2 and was subsequently stopped by a p53-mediated cell cycle arrest. We conclude, that stabilizing mutations of beta-catenin are rarely found in melanoma, because this type of mutation induces mitotic failure in melanocytic cells.

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Neuropilin 1 protects keratinocytes from UVB-induced apoptosis through regulation of Bcl-2

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Neuropilins (NRP1 and NRP2) are transmembrane receptors which act on endothelial cells and neurons to regulate angiogenesis and nerve outgrowth. They are able to bind to members of the VEGF family as well as to secreted class 3 semaphorins. It has been shown that NRP1 is also expressed and regulated on keratinocytes, but its function in the epidermis is still unclear.

To elucidate the role of epidermal NRP1 *in vivo*, we generated epidermis-specific neuropilin 1 deficient mice. These mice are viable and do not display obvious skin or hair defects. But we could demonstrate that deletion of epidermal NRP1 leads to increased apoptosis after UVB irradiation *in vitro* and *in vivo*. There is a significant increase of active caspase three positive cells in the epidermis of K14Cre-NRP1 (-/-) mice 24 h after irradiation. By Western Blot analysis we could show that NRP1 controls the expression of Bcl-2, a pro survival member of the Bcl-2 family. After irradiation the amount of Bcl-2 decreases in NRP1 deficient keratinocytes *in vitro* and *in vivo*. The amount of DNA damage detested in irradiated skin of control and NRP1 deficient animals is equal. Therefore, DNA repair mechanisms do not seem to be disturbed. We conclude that neuropilin 1 is dispensable for normal skin development but has an important anti-apoptotic role in UVB response, where NRP1 protects keratinocytes from apoptosis through modulation of Bcl-2.

P284

Fibroblast-induced mast cell differentiation promoted by kit-dependent and kit-independent pathways requires direct adhesion via VCAM-1 and $\alpha4\beta7$ integrin

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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 phenotypical change 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 differentiation. Since BMCMCs exhibited strong adhesion to Swiss albino 3T3 Fbs, we analysed the regulation of proliferation and differentiation of BMCMCs with

focus on the impact of this directed adhesion. Surprisingly, the proliferation of BMCMCs was markedly increased only if MC sunderwent direct cell-to-cell contact to Fbs, indicating that soluble Fb-derived factors such as soluble SCF are negligible in this context. Furthermore, the increase in histamine content and mast cell protease 4 (MCPT4) mRNA expression, i.e. indicators of differentiation towards CTMCs, in BMCMCs was dependent on direct adhesion as well. Most notably, MCs deficient for Kit, the receptor for the MC growth factor SCF, also showed a marked, albeit lesser increase in proliferation, histamine content and MCPT4 expression when cocultured with Fbs. These findings suggest an SCF/Kit-independent pathway for the modulation of MC biology by Fbs. However, the Kit-deficient BMCMCs showed no differences concerning their adhesion to Fbs as compared to wild type BMCMCs. Furthermore, we found that an interaction of Vascular Cell Adhesion Molecule 1 (VCAM-1) expressed by Fb and its ligand $\alpha4\beta7$ integrin on BMCMCs is largely responsible for the adhesion of both wild type and Kit-deficient BMCMCs. Moreover, we could show that the VCAM-1/$\alpha4\beta7$ itself does not induce BMCMC differentiation. Thus, our data show that BMCMC proliferation and differentiation towards CTMCs induced by Fbs is dependent on cell adhesion, in part induced by VCAM-1/$\alpha4\beta7$ interaction, but mediated by at least two separate pathways, one Kit-dependent and the other Kit-independent. The identification of membrane bound receptors other than Kit that induce MC proliferation and differentiation may provide interesting therapeutic targets for MC-driven diseases.

P285
Mast cells are critical for the limitation of thrombin-induced inflammation

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The serine protease thrombin is a major player in the coagulation cascade and is known to act via proteinase-activated receptors (PARs). Lately, it was shown that thrombin can have proinflammatory effects on different cell types and that PARs are expressed by many cells including mast cells (MCs), highly inflammatory cells that are located around blood vessels and critically contribute to skin inflammation. We, therefore, investigated the effects of thrombin on MCs. *In vitro* we examined murine bone marrow-derived mast cells (BMCMCs), peritoneal cultured MCs (PCMCs), and freshly isolated peritoneal mast cell (PMC). Thrombin stimulation resulted in a dose-depended degranulation of PCMCs and PMCs but not BMCMCs. Furthermore, we used specific PAR1 and PAR4 agonistic peptides and found that PCMC but not BMCMCs degranulate in response to these peptides. Quantitative PCR analyses of BMCMCs and PCMCs displayed expression of all three thrombin receptors, PAR 1,3, and 4 with the highest expression rate for PAR1. The intracutaneous injection of thrombin into ears of C57BL/6 mice resulted in a strong degranulation of MC assessed by quantitative histomorphometry. Thrombin injection also induced significant immediate inflammatory skin reactions in C57BL/6 mice. Surprisingly, this ear swelling was more pronounced in MC-deficient C57BL/6 Kit W-sh/W-sh mice, and reconstitution of C57BL/6 KitW-sh/W-sh mice with C57BL/6 BMCMCs normalized this effect. This suggests that MCs are necessary for the termination of thrombin-induced inflammatory responses. Interestingly, we also found that mast cell supernatant can degrade thrombin *ex vivo*. In summary, our data show that thrombin-induced immediate inflammatory skin reactions are controlled by cutaneous MC. Therefore, the regulation of mast cell function may be a promising target for treating injury-associated inflammation.

P286
Polarity in stem cells, disease and aging

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Adult somatic stem cells are central to homeostasis in tissues that present with a high cellular turnover like the skin, intestine and the hematopoietic system. It is thought that polarity is particularly important with respect to fate decisions upon stem cell division (symmetric or asymmetric) as well as for the maintenance of stem cell adhesion and quiescence (interaction with the niche). Consequently the failure to establish or regulate stem cell polarity might result in disease or tissue attrition. Members of the family of small Rho GTPases are known to exert an important role in regulating cell polarity. We will discuss in our presentation recent views and present novel data on the role of cell polarity in somatic stem cell function and aging, concluding that targeting cell polarity might be a novel approach to ameliorate or even revert aberrant somatic stem cell function.

P287
Von Willebrand factor promotes cutaneous inflammation

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Von Willebrand Factor (VWF) is a key player in hemostasis and is increasingly recognized as a proinflammatory protein. In previous studies we could show that VWF is an important regulator of neutrophil recruitment into the inflamed peritoneum. However, the role of VWF for cutaneous inflammation is yet unknown.

Irritant contact dermatitis and experimental vasculitis were studied both in mice treated with a VWF-blocking antibody and in VWF-/- mice. We observed in all methods a significant VWF dependent reduction of neutrophil recruitment to the inflamed skin (<50% of control, $n = 8$ each). In the anti-VWF antibody treated group this effect was dose-dependent. In line with these findings, inflammatory edema formation (measured via biopsy weight) was significantly decreased. While anti-VWF-antibody treatment did not affect bleeding time, we observed a significant reduction in endothelial permeability measured by tissue leakage of Evans blue.

Our data suggest that VWF regulates neutrophil recruitment to the skin by supporting the endothelial barrier function. Blocking VWF results in decreased neutrophil extravasation to the skin and therefore provides a novel therapeutic anti-inflammatory approach without interfering with the hemostatic system.

P288
Overlapping functions of kindlin-1 and -2 in epithelial cells: implications for phenotype modification in Kindler syndrome

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Kindlins are a novel family of intracellular adaptor proteins in integrin-containing focal adhesions. Kindlin-1 and -2 are expressed in the skin, but whether and how they cooperate in adult epithelial cells has remained elusive. Here, we uncovered the overlapping roles of kindlin-1 and kindlin-2 in maintaining epithelial integrity and show that the phenotype of kindlin-1 deficient cells can be modulated by regulatingkindlin-2 gene expression, and vice versa. The experimental evidence is provided by use of human keratinocyte cell lines which express either both kindlins, just kindlin-1 or kindlin-2, or none of them. Double deficiency of kindlin-1 and -2 had dramatic negative effects on focal adhesion formation and actin cytoskeleton organization, but also on cell adhesion, proliferation, directional migration and activation of $\beta1$ integrin, whereas deficiency of one kindlin only showed variable perturbation of these functions. Cell motility and formation of cell-cell contacts were particularly affected by lack of kindlin-2. These results predict that kindlin-1 and -2 can functionally compensate for each other, at least in part. The high physiological and pathological significance of the compensation was emphasized by the discovery of environmental regulation of kindlin-2 expression. UVB irradiation induced loss of kindlin-2 in keratinocytes and had negative effects on kindlin-1 deficient cells. This first example of environmental regulation of kindlin expression has implications for phenotype modulation in Kindler syndrome, a skin disorder caused by kindlin-1 deficiency.

P289
Efficacy of three new anti-wrinkle face care creams with urea and hyaluronic acid

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Introduction: Dry Skin is a promoting factor for the appearance of wrinkles on the skin, especially so if accompanied by underlying skin diseases like atopic eczema or psoriasis. Facial wrinkles are often considered as stigmatizing and thus may have profound effects on patient's self image, self-esteem, and sense of physical well-being. As a consequence, products counteracting these signs of ageing are in great demand.

We investigated the efficacy; skin compatibility and cosmetic performance of three new anti-ageing face creams especially formulated to suit the needs of dry to very dry skin (a day, a night and an eye care product) containing urea and hyaluronic acid as active ingredients.

Methods: The anti-wrinkle efficacy of the test products was assessed in a 6 weeks study with 43 female volunteers by dermatological assessment and standardized photographs. Additionally the moisturizing efficacy was also measured at baseline, 4 and 6 weeks. Furthermore, tolerability and cosmetic performance of the products were evaluated in an open study in 96 patients, partly suffering from atopic eczemaor psoriasis. The performance of the products under normal daily conditions was assessed in an in-use study with 358 volunteers with dry to very dry skin.

Results: For all tested products a good anti-wrinkle efficacy and marked increase in skin moisture content could be demonstrated. Furthermore, the products were well tolerated, even in patients with very dry skin suffering from atopic eczema or psoriasis and received favorable performance ratings.

Conclusions: The new range can be considered safe and effective for the anti-wrinkle treatment of dry skin sufferers even in the presence of atopic eczema or psoriasis.

P290
Results of *in vitro* and *in vivo* studies with a novel shave and after-shave regimen

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Background: Although male skin is considered to be more robust than female skin in various respects, regular shaving poses a significant risk of stress and irritation on skin, especially in patients suffering from inflammatory dermatoses. Therefore a novel shave and after shave skin care regimen containing antibacterial silver citrate and antiinflammatory licochalcone A was developed. Objectives: In the presented studies, the antibacterial properties of the formulations and the anti-inflammatory effect of the After Shave Balm were investigated.

Methods: *In vitro* suspension tests with staphylococci were conducted and rated after1, 3, 6, and 24 h. In a dermatological study skin irritation was induced by standardized mechanical abrasion with razor blades and skin recovery assessed with and without treatment with the After Shave Balm. In clinical studies the effect on skin condition and skin compatibility were investigated in atopic dermatitis and acne patients.

Results: Potent antibacterial efficacy was proven after 24 h in the suspension tests. The skin irritation study revealed good anti-inflammatory effects of the After Shave Balm and in the clinical studies the formulations were well tolerated and improved skin conditions were noted in most cases at the final investigation.

Conclusion: The novel product range proved to be effective in terms of antibacterial and anti-inflammatory properties and was well tolerated also by diseased skin. Namely for patients suffering from atopic dermatitis or acne, who are at special risk of skin problems like razor burns, cuts and nicks, the tested regimen offers effective relief.

P291
Dihydrodehydrodiisoeugenol enhances adipocyte differentiation and decreases lipolysis in murine and human cells

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During chronological aging the distribution of fat in the body changes: subcutaneous fat depots decrease whilst visceral fat depots increase. Starting in the face, the thickness of the adipose tissue decreases resulting among others in sunken cheeks. For this facial lipoatrophy as a normal process of aging different reasons are known: (i) the preadipocytes in the subcutaneous fat tissue of the skin lose the capacity to differentiate, (ii) tumor necrosis factor (TNF)-alpha is secreted in elevated levels by adipocytes of elderly with diverse effects on the fat storage capacity of adipose tissue by (i) enhancing lipolysis, (ii) inhibiting the differentiation of preadipocytes to adipocytes and (iii) maintaining the dedifferentiation of adipocytes. Adipocyte differentiation is mainly controlled by a transcriptional cascade involving peroxisome proliferator-activated receptor γ (PPARγ) and members of the CCAAT enhancer binding protein (C/EBP) family of transcription factors.

As *in silico* approaches had revealed that some neolignans present a PPARγ receptor ligand binding domain, the synthetic neolignan dihydrodehydrodiisoeugenol was assessed for its capacity to (i) increase adipocyte differentiation and to (ii) inhibit TNF-α mediated and/or basal lipolysis in a murine and a human cell culture system. For this purpose, the differentiation process of murine 3T3-L1 preadipoctyes was started at confluence and studied for another 10 days using a standard differentiation protocol including a hormone mix (HM) containing insulin, dexamethasone and isobutylmethylxanthine. The induction of the differentiation process was controlled for upregulation of differentiation specific transcription factors such as PPARγ2 andC/EBPα, but also for induction of fatty acid binding protein 4 (FABP4) and adiponectin and for down-regulation of the preadipocyte marker pref1 using real time PCR. Dihydrodehydrodiisoeugenol significantly upregulated the differentiation markers and resulted in a 30% increase of lipid accumulation as compared to the HM control. In addition, dihydrodehydrodiisoeugenol was able to reduce basal and TNF-α induced lipolysis detected as release of free glycerol in a dose-dependent manner.

In primary human subcutaneous preadipocytes, an age-appropriate hormone mix with regards to insulin and dexamethsasone was used for induction of adipocyte differentiation. Under these conditions, dihydrodehydrodiisoeugenol was able to significantly increase lipid accumulation above HM controls. In addition, dihydrodehydrodiisoeugenol also induced upregulation of FABP4 and adiponectin mRNA.

Moreover, dihydrodehydrodiisoeugenol was able to partially replace a PPARγ agonist such as troglitazone during the differentiation process resulting in a significant increase of lipid accumulation. In an *in vitro* approach, dihydrodehydrodiisoeugenol presented PPARγ binding capacity underlining its role as at least partial PPARγ agonist. Finally, the compound was also able to inhibit basal lipolysis in mature human subcutaneous adipocytes. Taken together, these *in vitro* data indicate that dihydrodehydrodiisoeugenol might be well suited to over comelipoatrophy by a dual mechanism that is increasing adipocyte differentiation and inhibition of lipolysis.

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Two-dimensional luminescence imaging of pH *in vivo*

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Luminescence imaging of biological parameters is an emerging field in biomedical sciences. Tools to study two-dimensional (2D) pH distribution are needed to gain new insights into complex disease processes, such as wound healing and tumor metabolism. In recent years, luminescence-based methods for pH measurement have been developed *in vitro*. However, for *in vivo* applications biocompatibility and reliability under varying conditions have to be ensured. Here we present a referenced luminescent sensor for 2D high-resolution imaging of pH *in vivo*. The sensing schemeis based on luminescence imaging of fluoresceinisothiocyanate (FITC) and ruthenium(II)tris-(4,7-diphenyl-1,10-phenanthroline) [Ru(dpp)3]. To create a biocompatible 2D sensor, these dyes were bound to or incorporated into microparticles (amino cellulose, polyacrylonitrile) and particles were immobilized in polyurethane hydrogel on transparent foils. We demonstrate sensor precision and validity by conducting *in vitro* and *in vivo* experiments, and show the versatility in imaging pH during physiological and chronic cutaneous wound healing in humans. Implementation of this technique may open new vistas in wound healing, tumor biology and other biomedical fields.

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Characterization of the protective immunmodulation of probiotic bacteria in localised Candidiasis

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The commensal yeast *Candida albicans* is present in about 50% of the oral cavity of healthy humans and the main-agent of fungi-caused diseases in humans. Usually, *C. albicans* is part of the normal microflora of the oral cavity, the intestine and the vagina and can become pathogenic when the host immune system is weak. Several probiotic Lactobacillus species are described, that exert inhibiting and/or protective effects on *C. albicans* and other infections *in vivo* and *in vitro*. Therefore we choose *L. rhamnosus* GG to investigate the effect of this species on localised *C. albicans* infections and the mucosal innate immune system.

Using a model system of localised candidiasis based on reconstituted human oral epithelium (RHE) we investigate a number of different aspects of host/Candida interactions as well as the effects on the host immune response.

Our results indicate a protective role for *L. rhamnosus* GG (LGG) in our model. Candida infected RHEs treated with Lactobacilli showed significantly lower levels of lactate dehydrogenase (LDH), used as a marker of cell damage, compared to LDH-levels of untreated RHEs. Protection can also be confirmed by light microscopy where epithelium with co- cultured Candida and Lactobacilli resemble untreated controls whereas epithelium cultured with Candida alone is strongly damaged. Furthermore, LGG seems to decrease the proinflammatory cytokine response of the RHEs towards Candida infection. The same effects are observable using a monolayer system based on keratinocytes isolated from the RHE.

Using this monolayer-based system, preliminary results revealed less adhesion and reduced invasion of *C. albicans* to the keratinocytes in presence of LGG.

Taken together, our results indicate that LGG offers protection against oral *C. albicans* infection by preventing adhesion and invasion of the fungi to the epithelium on the one hand but also by modulation of the host immune response on the other hand.

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For both ammonium ions of the skin surface and transcutaneous carbon dioxide partial pressure a significant relation to skin surface pH and trans epidermal water loss can be found when analyzing raw data as well as raw data standardized to ambient relative humidity

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While the acid reaction of the skin surface has been well described, the regulation of skin surface pH is less well characterized. Assessing molecules related to pH may be of interest to obtain new insights. Two such molecules are ammonium ions and carbon dioxide. Since ammonium ions may have the gas ammonia as precursor and carbon dioxide is a gas, studying the relation of both molecules to skin surface pH also should include the assessment of trans epidermal water loss (TEWL) as a measure for skin permeability. Therefore, the aim of the present study is to compare ammonium ions and transcutaneous carbon dioxide partial pressure (tcPCO2) with skin surface pH and TEWL using correlation analysis. Since in particular TEWL canbe regarded as very sensitive to the ambient relative humidity (RH) the results are analyzed as raw data as well as after standardization (ST) to RH.

Overall, 30 female volunteers aged 18–30 were included into the study. The areas of measurement were forearm (FA) and forehead (FH). All assessments were performed at a room temperature of 20–22°C. RH was monitored for each volunteer. The ammonium ions were determined *ex vivo*. To gain a sample a skin area of 22 mm in diameter was rinsed with 6 ml water. Quantification of the ions in the sample was performed by means of the Berthelot reaction using a kit that is commercially available. The tcPCO2 as well as skin surface pH and TEWL were assessed non-invasively using devices that are commercially available. For all parameters mean and standard deviation were calculated for the raw data and for the values standardized to RH. Also, for both groups correlation analysis was performed.

The mean absolute values and the standard deviations calculated showed the expected amplitude and range for all parameters, while RH was 57.2 ± 5%. The most pertinent results from the correlation analysis using the raw data were a significant negative correlation between ammonium and pH on the FA (r = -0.381; P = 0.038) and a significant correlation between ammonium and TEWL on the FH (r = 0.428; P = 0.018). After ST to RH a significant correlation between ammonium and TEWL (r = 0.496; P = 0.005) was found on the FH. Furthermore, after ST to RH a highly significant correlation between tcPCO2 and pH (r = 0.674; P < 0.001) accompanied by a significant correlation between pH and TEWL (r = 0.427; P = 0.019) was found on the FA. Also after ST to RH, a significant correlation between tcPCO2 and pH (r = 0.493; P = 0.006) accompanied by an almost significant correlation between tcPCO2 and TEWL (r = 0.347; P = 0.060) was found on the FH.

In general, ST of the values to RH has significant influence on the results. The ST to RH might reflect in particular the influence of TEWL and skin permeability. With respect to ammonium, the inverse relation between ammonium and pH found on the FA without ST to RH indicates that the ammonium ions might have reached the skin surface already as ammonium ions for example via sweat. The particular strong relation to TEWL found on the FH after ST to RH, however, suggests that the ammonium ions in this region might also significantly be due to the diffusion of the gas ammonia. With respect to the tcPCO2, the appearance of significant results when standardizing the values to RH indicates that skin permeability has the expected effects. The positive correlation to pH further suggests that loss of carbon dioxide may increase the pH of the skin surface showing a certain similarity to sweat physiology. Further studies are required to investigate the influence of RH in detail.

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Black tattoo inks in skin – a hazard chemical cocktail?

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Background: About 10 % of population in Germany is tattooed. The tattoo colorants predominantly consist of black inks or coloured azo pigments. Black tattoo inks mainly consist of Carbon Black (CB) that is usually produced by imperfect combustion. CB is known to act as a significant strong sorptive phase for poly cyclicaromatic hydrocarbons (PAHs). There is currently no information available whether PAHs may stay in skin together with Carbon Black. In addition, due to missing control and regulation of tattoo inks, the black inks may also contain other impurities.

Objective: To assess the health risk of tattooing, it is firstly important to investigate whether tattoo particles in skin contain harmful PAHs. Secondly, it is important to identify and quantify impurities in black tattoo inks.

Material and Methods: We firstly developed an extraction method to recover PAHs from human skin. We secondly developed extraction method to identify and quantify black ink impurities. For both extractions, we used a mixture of benzene/acetone and applied heat, ultrasonic and centrifugation. Chemical analysis and quantification were performed by using HPLC-DAD and GC-MS with internal standard procedure.

Results: Using human skin suspensions, we successfully recovered a selection of 20 PAHs, which are listed as priority pollutants by the US-EPA or European Commission because of their high toxicity and as probable human carcinogens. The recovery rate was higher than 90 % for all PAHs. Then, we extracted impurities from 20 commercial black tattoo inks. We identified eight different substances such as softener(e.g. Dibuthylphthalate) that are forbidden to be used in cosmetics. The softener Dibuthylphthalate is usually applied as emollient/softener in PVC production, gums and printers ink and is presently strictly forbidden in toys, cosmetics and nail polish because of its carcinogenic and teratogenic activity. We also found toxichalogen-compounds like HCBD (1,1,2,3,4,4 hexachloro1,3-Butadien).

Conclusion: In light of the successful PAHs recovery, an extraction of PAHs from tattooed skin will be performed in the near future. Moreover, black inks may contain a variety of harmful impurities that are injected into skin during tattooing. Beside many case-reports in the medical literature, a nationwide survey in German-speaking countries shows that up to 9% of tattooed people have transient or even persisting health problems after tattooing. Black ink is the most frequent tattoo colorant (60 % of tattooed individuals). Our results provide first indication that these health problems might be correlated to the chemistry of the used black tattoo inks.

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The impact of beta1 integrin signalling on adult human hair follicle epithelial progenitor cell viability

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Integrins are part of the human epithelial stem cell niche and control via cell–cell- and via-cell-matrix interactions important biological processes for cell growth, differentiation and polarity. Recently, we could show that the beta1 integrin activating antibody 12G10 has a positive effect, e.g. on hair shaft elongation of organ cultured human hair follicles.

Here, we speculated now that this proliferative effect could be due to activated beta 1 integrin positive epithelial stem cells within the HF outer root sheath. Our aim was to explore if there is a direct influence in regulating epithelial HF progenitor cells via thebeta1 integrin receptor by supplementation of the receptor activating 12G10 to HF organ cultures. To follow-up the influence of 12G10 on HF epithelial stem cells, we used Dispase pretreated, isolated HFs which were subsequently transfected with a human Cytokeratin15 (K15)-promoter-driven GFP-expression system (K15-GFP)allowing the demarcation of K15+ HF progenitors *in situ*. The K15-GFP transfected HFs were organ-cultured for 3 days and were treated with 12G10 directly after transfection. Differences in the K15-GFP signal were measured by fluorescence analysis and by counting the number of single K15-GFP+ cells within the HF bulge and bulb region every 24 h during the culture period. Parallel, HFs were embedded, cryosectioned and quantified the immunofluorescence reactivity of endogenous K15 protein.

By that, we discovered a tremendous increase of K15-GFP signals with the highest peak on the second day followed by a decrease on day 3 in the HF bulge and in the bulb regardless the presence of 12G10. However, the beta1 integrin activating12G10-HF group showed a higher K15-GFP intensity than the untreated group. The single number of K15-GFP+ cells in the bulb region does not vary during the culture period whereas in the bulge we have three times more single K15-GFP+ cells on day 2 compared to day 1 in 12G10 treated or non-treated HFs. Surprisingly, K15 immunoreactivity in the cryosections was only found in the bulge and in the lower HFbut not in the bulb. In summary, the activating 12G10 antibody increases theK15-GFP intensity. These findings could be in line with assumed epithelial stem cell activation to their committed progeny (e.g. transient amplifying cells).

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Biodegradable poly lactic acid particles for transcutaneous drug delivery and skin cell targeting

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In previous studies with barrier-disrupted human and mouse skin models, we have shown that antigen presenting cells can be targeted using different types of particulate carriers, e.g. polystyrene particles (PS) and modified vaccinia Ankara (MVA). In this study, we used biodegradable poly lactic acid (PLA) particles as well as PS particles for the transcutaneous delivery of the HIV-1 p24-peptide as well as the targeting and activation of Langerhans cells (LCs).

In *in vitro* experiments we found that both PLA and PS particles were internalized by LCs, delivered the surface-adsorbed HIV-p24 peptide and induced the expression of maturation markers. Upon topical application on the skin surface, particles preferentially accumulated in hair follicles where they released the adsorbed peptide. Peptide-loaded PS particles were detected in LCs isolated 16 h after particle topical application on excised human skin. Up-regulation of CD80 and CD83 along with adown-

regulation of CD1 a surface molecules was observed after treatment with both p24-loaded PS and PLA particles. Thus, both particle types allowed for the delivery of HIV-1 p24 peptide and the modulation of skin immune system. Both mechanisms, i.e. transcutaneous delivery of particles and particle-based delivery of adsorbed antigens, may open interesting new transcutaneous vaccination strategies and skin cell targeting.

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IL-24 plays a key role in cutaneous wound healing via signaling throughIL-22R1/IL-20R2 receptor complex

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Cutaneous wound healing is a complex regenerative and immunological process, and its disturbance represents a great medical problem. In the past, we demonstrated that the novel IL-10 cytokine family members IL-22 and IL-20 play a major role in psoriasis and skin homeostasis. Here, we studied whether these cytokines as well as another closely related cytokine of this family, IL-24, play a role in cutaneous wound healing. Surprisingly, using an *in vivo* mouse model, IL-20 was almost constantly expressed upon wounding and following healing process, whereasIL-22 was not expressed at all. However, IL-24 was highly upregulated in the early phase of wound repair. The major sources of IL-24 appeared to be T cells and keratinocytes. IL-24 shares the IL-22R1 receptor subunit with IL-22 and IL-20 for mediating its biological activity. Importantly, mice lacking IL-22R1 (IL-22R1-/-) compared to corresponding wild-type mice (WT) showed a delayed wound closure starting in the early phase of the healing process. Further studies identified keratinocytes, but not dermal fibroblasts, endothelial cells, melanocytes, or subcutaneous adipocytes as being targets of IL-24 action. The IL-24 treatment of human keratinocytes from both conventional cultures and three-dimensional human epidermis model regulated the expression of many differentiation-associated genes and chemokines. This study suggests that the IL-24/IL-22R1 system plays a key role in the inflammatory phase of cutaneous wound healing.

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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 ineccrine 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 48 mer 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 that DCD-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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