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Allergy

P001

Novel insights into mastocytosis-associated osteoporosis: mast cells in indolent systemic mastocytosis produce bone cytokines

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Mastocytosis is characterized by pathologic accumulation of mast cells in different tissues, particularly in skin and bone marrow. In patients with systemic mastocytosis involving the bone marrow, more than half of the patients show osteopenia or osteoporosis. In the past decade, a series of new cellular and molecular players have been identified that coordinate the balance between bone-forming osteoblasts and bone-degrading osteoclasts in a highly complex manner.

In the present study, we sought to investigate the pathophysiology of skeletal involvement in mastocytosis. In particular, we aimed to explore whether key cytokines of bone metabolism, such as receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG), sclerostin (SOST) and dickkopf-1 (Dkk-1) are altered in the serum of patients with systemic mastocytosis and whether mast cells are able to produce these cytokines.

Our results show that RANKL, OPG and SOST are significantly increased in the serum of patients with indolent systemic mastocytosis associated with osteopenia or osteoporosis. In contrast we did not find altered levels of Dkk-1. Performing immunohistochemistry and immunofluorescence of bone marrow sections from patients with systemic mastocytosis, we observed clear colocalization of RANKL- and OPG-positive cells with tryptase-positive mast cells, demonstrating that mast cells in mastocytosis produce RANKL and OPG. Furthermore, we detected significant levels of RANKL, OPG and SOST in supernatants of mast cell lines.

Thus, we show for the first time that mast cell-derived RANKL, OPG and SOST are altered in systemic mastocytosis. Our data provide a rationale for investigating RANKL, OPG and SOST as potential diagnostic markers of mastocytosis-related osteoporosis. Moreover, our results suggest exploring these cytokines as novel therapeutic targets in mastocytosis with skeletal involvement.

P002 (O14)

Activated regulatory T cells strongly inhibit allergen-induced colitis in a PBMC-engrafted murine model of allergy

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Recently, we have developed a humanized mouse model of allergen-induced IgE-dependent gut inflammation in PBMC-engrafted immunodeficient mice. In the present study we investigated the role of regulatory T cells (Treg) in this model. Therefore, NOD-scid-$\gamma c-/-$ mice were injected intraperitoneally with human PBMC from allergic donors together with the respective allergen or with NaCl as control in the presence or absence of different concentrations of CD4+CD25+ Treg of the same donor. After an additional allergen boost 1 week later, mice were challenged with the allergen rectally on day 21 and gut inflammation was monitored by a high resolution video mini-endoscopic system. Allergen-specific human IgE in mouse sera, which was only detectable in PBMC plus allergen-treated mice, was strongly inhibited by co-injection of Treg at a ratio of at least 1:10. The presence of Treg also reduced allergen-specific proliferation and cytokine production of human CD4+ T cells recovered from spleens at the end of the experiment. Furthermore, the allergen-induced endoscopic score evaluating translucency, granularity, fibrin production, vascularity, and stool after rectal allergen challenge was significantly decreased by Treg. Activation of Treg prior to injection further increased all inhibitory effects. These results demonstrate that allergen-specific gut inflammation in human PBMC-engrafted mice can be avoided by enhancing the numbers of autologous Treg in these mice which is of great interest for therapeutic intervention concerning allergic diseases of the intestine.

P003

Development, validation and initial results of the urticaria control test – a novel patient reported outcome instrument for assessing urticaria control

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Background: Chronic urticaria is a frequent and debilitating skin disease. Assessing disease activity of these patients is a major problem since urticaria symptoms commonly fluctuate considerably from day to day. Therefore, the clinical picture of a patient at the time of presentation at his/her treating physician is only rarely representative for the actual current disease status. As of yet, the only reliable way to assess current disease activity is the prospective determination of symptoms over several consecutive days with the Urticaria Activity Score (UAS). However this tool has some major limitations, e.g. it works only as a prospective instrument and it is only designed for patients with chronic spontaneous urticaria but not other inducible urticaria subtypes.

Objective: To develop and validate a novel patient reported outcome instrument to retrospectively assess urticaria control, the Urticaria Control Test (UCT), in patients with all types of chronic urticaria (spontaneous and inducible forms).

Methods: Potential UCT items were developed by using established methods (literature research, expert and patient involvement). Subsequently, item reduction was performed by a combined approach applying impact and regression analysis. The resulting UCT instrument was then tested for its validity, reliability and screening accuracy.

Results: A 4-item UCT with a recall period of 4 weeks was developed based on 25 potential UCT items tested in 508 chronic urticaria patients. A subsequent validation study with the final 4-item UCT in 120 chronic urticaria patients demonstrated that this new tool exhibits good convergent and known-groups validity as well as excellent test-retest-reliability. In addition, the screening accuracy to identify urticaria patients with insufficiently controlled disease was found to be high.

Conclusions: The UCT is the first valid and reliable tool to assess disease control in chronic urticaria (spontaneous and inducible) patients. Its retrospective approach and simple scoring system make it an ideal instrument for the management of chronic urticaria patients in clinical practice.

P004

Characterization of the effects of Omalizumab in ‘real life’ difficult-to-treat chronic urticaria

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Omalizumab (anti-IgE) therapy has been shown to be effective and safe in chronic urticaria (CU) in placebo-controlled clinical trials but real life clinical data are scarce. Here, we report about a retrospective clinical analysis, assessing responder rates, optimal dosage, response to up-/downdosing, time to relief of symptoms, rates of return and time of relapse as well as changes in blood parameters and responses to skin provocation tests after omalizumab administration in up to 51 CU patients, 20 with chronic spontaneous urticaria (CSU) alone, 21 with different forms of chronic inducible urticaria (CindU) and 10 with both. Omalizumab treatment led to complete remission in 83% of CSU and 70% of CindU patients. When starting with 150 mg omalizumab 4 weekly, only 2/15 CSU and 7/17 CindU patients required updosing to achieve complete remission. In CSU, 57% of complete responses occurred within week 1, all on the first day. Relapses were 2–8 weeks in all but six patients, where they were <4 months. In some patients who showed complete response, treatment had to be stopped, for example because the insurance would only cover the costs for the treatment for a certain time period. Since all of these patients again developed severe symptoms, retreatment with omalizumab was eventually started. After initiation of retreatment, all patients showed again a rapid and complete response. None of the patients reported relevant adverse events during omalizumab treatment and retreatment. To identify possible predictors and markers of response to omalizumab, we assessed total serum IgE, serum tryptase levels, and circulating basophil numbers performed autologous serum skin test and skin prick tests to histamine and codeine in some of the patients before and after treatment with omalizumab. In CSU but not CindU patients, an increase in basophil numbers after omalizumab therapy was observed (from 0.01 to 0.025 cells per nl, $P > 0.01$), all other parameters, including all provocation tests, did not correlate with omalizumab efficacy. Taken together, clinical experience from more than 1250 injections in 51 patients over 4 years indicates that omalizumab is a rapidly acting, highly effective and safe drug in CSU and CindU patients. Our observations in a real life clinical setting support the recommendation of current EAACI/GA2LEN/EDF/WAO guideline for the management of urticaria to use omalizumab to treat urticaria patients.

P005 (O02)

A beneficial role for immunoglobulin E in host defense against honeybee venom

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Allergies are widely considered to be misdirected type 2 immune responses, in which IgE antibodies are produced against any of a broad range of seemingly harmless antigens. However, components of insect venoms also can induce allergic sensitization and development of specific IgE antibodies, which bind to the high affinity IgE receptor, FcεRI, on tissue mast cells (MCs) and blood basophils, priming them to release mediators of allergy and anaphylaxis upon subsequent venom exposure. MCs also can be activated directly by certain venoms, in the absence of specific IgE, and work in mice indicates that innate functions of MCs, including degradation of venom toxins by MC-derived proteases, can enhance host resistance to the venoms of certain arthropods and reptiles. Here, we hypothesized that acquired type 2 immunity against venoms also can enhance host defense. Injection of mice with amounts of honeybee venom similar to that which could be delivered in one or two stings resulted in the development of a specific type 2 immune response which increased the resistance of the mice to subsequent challenge with potentially lethal amounts of the venom. Using various transgenic mouse strains including Fcer1$\gamma-/-$, Fcer1$\alpha-/-$ and IgE-deficient Igh-7$-/-$ mice we further show that IgE antibodies and the high affinity IgE receptor, FcεRI, were essential for such acquired resistance to honeybee venom. Taken together, our data indicate that one function of IgE, which is best known for its role in allergic reactions, is to protect the host against noxious substances.

P006 (O25)

Opposing role of IL-31 in human skin biology

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Inflammatory skin diseases like atopic dermatitis (AD) are characterized by an impaired skin barrier, increased allergen priming, as well as decreased hydration of stratum corneum and resistance to staphylococcus and often severe itching. The expression of the cytokine IL-31 is increased in skin lesions of AD patients and correlates with disease severity. In previous studies we demonstrated that IL-31 is capable to alter skin barrier morphology *in vitro* by interfering with keratinocyte proliferation and differentiation. Aim of this study was to investigate the functional impact of IL-31 on skin barrier and to determine the -so far unknown- physiological role of IL-31 in healthy human skin. Organotypic 3-D skin models were pretreated with IL-31 and barrier function was studied either by measuring the penetration of fluorescent labeled grass pollen allergens applied to the surface or biotin applied to the dermal side of the skin models. The IL-31 treated models showed a remarkable reduced barrier function both of the stratum corneum and the epidermal desmosomal junctions. To follow up these results we used a human cell-sorted skin equivalent placed on the back of SCID mice. In this *in vivo* model we identified the influence of IL-31 on epidermal morphology and decreased filaggrin expression leading to enhanced transepidermal water loss. Surprisingly, we identified the IL-1 cytokine network as a key downstream effector of the IL-31/IL-31 receptor axis, with Anakinra, an IL-1 receptor antagonist, rescuing the IL-31 effects on skin differentiation and barrier formation. This cytokine network downstream of IL-31 is also responsible for an increased expression of a series of antimicrobial peptides (AMP) like RNase7, the human beta defensins (hBD) hBD-2 and hBD-3 as well as members of the S100 calcium binding family S100A7, S100A8 S100A9 and S100A12. Titration studies revealed that low doses IL-31 are capable to increase AMP expression treatment without disruption of the skin barrier. In conclusion, our data suggests a dual function of IL-31 in the skin, depending on the cytokine concentration. Only high concentrations of IL-31 (100–10 ng/ml) were capable to weaken the skin barrier whereas low concentrations of IL-31 (1 ng/ml) were sufficient to stimulate via the IL-1 cytokine network the expression of AMPs. These findings indicate a role of IL-31 in the host defense of healthy human skin and must be considered during therapeutic targeting of IL-31.

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P007

Effects of pollen-derived non-protein substances on the allergic immune response *in vivo*: roles of adenosine, PALMs and neuroreceptors

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Background: As previously shown, allergen-free fractions of aqueous pollen extracts (APE) contain eicosanoid-like lipids, the pollen-associated lipid mediators (PALMs), as well as adenosine. Both substance classes activate and modulate human immune cells *in vitro*. In this project, the relevance of non-allergenic pollen-derived compounds on the human allergic immune response was assessed in two experimental *in vivo* situations, skin prick testing and nasal provocations.

Methods: A protein-free, low molecular weight fraction of APE (APE < 3 kDa), lipid extracts of APE and adenosine were tested for aggravating potential in skin prick tests with birch and grass allergens. Clinical end-point was wheal size. Birch and grass pollen-allergic patients were nasally provoked with an allergen fraction of APE either alone or together with APE < 3 kDa. Experimental readouts were proinflammatory cytokines and total IgE in nasal secretions and measurement of nasal secretion production. Clinical end-points were rhinomanometry and differential symptom scores. Whole trancriptome analysis was performed from nasal epithelial scratch biopsies.

Results: In skin prick tests, birch and grass allergen in combination with APE < 3 kDa induced larger wheals than the allergen alone. The effect of adenosine was either aggravating or inhibitory, depending on the patient tested. However, a lipid extract of APE, containing PALMs, proved clearly to excert adjuvant activity. In nasal provocations, allergic patients challanged with allergen plus APE < 3 kDa produced higher amounts of secretion, showed elevated levels of IL-8 and IgE in nasal secretions and reported stronger ocular symptoms, sneezing and rhinorrhea. Nasal obstruction was the same in both study groups. Microarray data suggests an upregulation of neuroregulatory receptors, e.g. serotonin receptor, in the APE < 3 kDa group.

Conclusion: APE < 3 kDa exerts an aggravating effect on the cutaneous as well as the nasal allergic response to pollen allergen. In the skin, the role of adenosine is conflicting due to inter-individual differences, whereas PALMs clearly enhance the allergic response. In the nose, neuromodulatory receptors might play a role for the aggravation of allergic symptoms.

P008

Elevated specific IgE against MGL_1304 in sera of patients with atopic dermatitis and cholinergic urticaria

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Background: MGL_1304 secreted by Malassezia globosa is contained in human sweat and induces histamine release from basophils in patients with atopic dermatitis (AD) at a high positive rate. The aims of this study were to establish the enzyme-linked immunosorbent assay (ELISA) measuring specific immunoglobulins against MGL_1304 and to investigate the levels of these immunoglobulins in sera of patients with various allergic diseases.

Methods: Purified MGL_1304 from human sweat (QRX) and recombinant MGL_1304 (rMGL_1304) were prepared for ELISA. To quantify the amount of MGL_1304-specific immunoglobulins, the standard serum was created by pooling sera of 20 patients with AD whose basophils released histamine in response to QRX. A monoclonal antibody which exhibited the highest neutralizing ability against QRX was established as Smith-2, and used as a capture antibody for the assay of QRX-specific IgE. A total of 156 subjects (normal controls (n = 23), AD (n = 63), cholinergic urticaria (CU) (n = 24), bronchial asthma (n = 32), and allergic rhinitis (n = 14)) were enrolled in this study.

Results: ELISA methods to quantify the specific IgE, IgG and IgG4 against MGL_1304 in sera were successfully established. Levels of QRX-specific IgE in sera of patients with AD and CU were significantly higher than those of normal controls. Moreover, the levels of QRX-specific IgE and rMGL_1304-specific IgE in patients with AD were significantly correlated with their disease severities.

Conclusions: These ELISA methods to quantify the specific immunoglobulins against MGL_1304 are easy and useful means to assess allergy to MGL_1304. MGL_1304 contained in sweat is an important antigen for patients with AD and CU.

P009

Differentiation of regulatory and memory T cells during wasp venom immunotherapy

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Background: It has become clear that regulatory T cells can be divided into several distinct subsets with unique functional and homeostatic properties. Upon subcutaneously administered wasp venom immunotherapy (VIT) the induction of allergen-specific Treg are believed to play a pivotal role in promoting long lasting immune tolerance to the causative allergen in part by balancing the antigen-specific activation of Th1 (IFN-γ)- and Th2 (IL-4) cells. However, whether high-dose wasp VIT influences the phenotypical specialization of Treg, Th1 and Th2 cells with respect to tissue-specific trafficking and function is largely unknown.

Objective: We investigated the peripheral trafficking and functional capacity of CD4+CD25+Foxp3+CD45RO+ memory Treg and CD4+CD25−CD45RO+ memory T cells in the course of VIT.

Patients and methods: Treg and memory T cells of freshly isolated peripheral blood mononuclear cells (PBMC) of 9 wasp venom-allergic patients eligible for wasp VIT were analysed by flow cytometry for surface expression of lymphoid and non-lymphoid chemoattractant receptors, namely skin-homing chemokine receptors cutaneous lymphocyte antigen (CLA), CCR4 as well as CCR6, gut-homing integrin αE(CD103)β7 and lymph node seeking CCR7 and L-selectin CD62L, respectively. In parallel, cells were studied for the Th1-associated expression of CXC chemokine receptor 3 (CXCR3) and of CCR5, also a marker reflecting education of Treg in inflamed tissues. Furthermore, maximal Th1/Th2-cytokine secretion of peripheral T cells was assessed by anti-CD3 stimulation.

Results: In confirmation of our earlier results, VIT promoted the induction of CCR7/CD62L coexpression on peripheral memory Treg but not on conventional memory T cells 1 month after starting VIT, indicating a recirculation to secondary lymphoid organs. Interestingly, CCR5 was also expressed at significant higher levels on circulating Treg, presumably promoting the injection site-directed education, whereas one of the most important skin-homing receptors, CCR4, remained at a constant high level. Analysis of the Th1-associated receptors yielded a significant induction of CCR5 and CXCR3 on memory T cells, whereas the Th2 marker CCR4 remained unchanged.

The characterization of peripheral trafficking markers was paralleled by a profound increase in IFN-γ (Th1) and IL-10 (Treg) production by unfractionated CD3-stimulated PBMC after 1 month of therapy.

Conclusions: VIT does not only induce the trafficking of peripheral Treg into secondary lymphoid organs but also appears to promote Treg education and function as assessed by CCR5 expression and IL-10 secretion. Finally, the enhanced expression of Th1-chemokine receptors CXCR3 and CCR5 on memory T cells underlines that VIT promotes strong IFN-γ responses accompanied by IL-10 producing Treg.

P010

Allergen specific tolerance induction is independent of interleukin 10 signaling in T cells, B cells or neutrophils/monocytes

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Human studies suggest that allergen immunotherapy leads to regulatory immune responses that actively suppress the development of allergic inflammation. Interleukin (IL)-10 production by allergen-specific T cells has been suggested as one of the main regulatory mechanisms. This was supported by *in vivo* studies in mice in which treatment with IL-10 rReceptor (IL-10R)blocking antibody abrogated the beneficial effects of immunotherapy. In contrast to existing concepts that highlight the role of T cell derived IL-10, we have previously demonstrated that in mice with a T cell specific inactivation of the IL-10 gene (IL-10FL/FL CD4-Cre+) tolerance induction is unaffected, while in mice that carry an IL-10 deletion in all hematopoietic cells (IL-10FL/FL vav-Cre+) tolerance induction was impaired, suggesting that hematopoietic sources of IL-10 other than T cells contribute to the beneficial effect of immunotherapy. In the present study we address the cellular targets of IL-10 in the process of tolerance induction by using mice with a cell type specific inactivation of the IL-10R (IL-10R) gene generated by Cre/loxP-mediated recombination. Ovalbumin (ova) sensitized mice were treated with three subcutaneous ova injections on alternate days for tolerance induction. One week later mice were challenged by ova inhalation and subsequently allergen specific antibody and cytokine responses as well as allergen induced airway inflammation was analyzed. Tolerance induction was effective in the suppression of allergen induced airway inflammation in wildtype mice but not in IL-10R null mutants (IL-10RFL/FL Cre deleter+), confirming the involvement of IL-10R in tolerance induction. In contrast, in mice that lack IL-10 signaling specifically in T cells (IL-10RFL/FL CD4-Cre+) the degree of tolerance induction was comparable to that of Cre negative littermate controls both displaying strongly reduced eosinophilic infiltration into the bronchoalveolar space and reduced Th2 responses to allergen specific restimulation. To address the role of IL-10 signaling in B cells and neutrophils/monocytes we made use of mice with lineage specific deletion of the IL-10R. Tolerance induction in mice with a B cell specific (IL-10RFL/FL CD19-Cre+) as well as in mice with a neutrophil/monocyte specific (IL-10RFL/FL LysM-Cre+) deletion of the IL-10R was also comparable to that of Cre negative littermate controls. In summary, our results show that in the murine model of allergen induced airway inflammation direct effects of IL-10 on T cells, B cells or neutrophils/monocytes are not critical for tolerance induction. Thus different cellular targets of IL-10 are likely to be involved in the benefical effects of allergen specific tolerance induction.

P011

Generalized phototoxic dermatitis due to ingestion of rue tea (*Ruta graveolens* L.)

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Introduction: The phototoxicity of *Ruta graveolens* L. has been mainly attributed to furocoumarins and to the photosensitizing properties of furoquinoline alkaloids. Although both components demonstrate similar mechanism of action, the alkaloids also induce monoadducts and form cross-links with the DNA in the presence of ultraviolet light. Dermatic reactions have been reported following high concentrated rue infusion and subsequent sun exposure, however, generalized reactions due to oral consumption of rue extracts has not been reported yet.

Methods and results: A 50-year-old woman presented with increasing discomfort due to generalized skin burnings following burns, 48–72 h sun exposure and systematic ingestion of rue tea. She reported burning sensation and limited mobility due to intense pain. The clinical examination revealed erythema, edema with tense vesicles,blisters and bullae of the whole skin in the exposed areas. The central and lower areas of her back and legs demonstrated epidermolytic lesions. Initial treatment was started with with high dose prednisolone intravenously and was followed by topical steroids, antiseptic ointments, wet dressings and analgesics. Clinical symptoms have resolved within 3 weeks leaving residual hyperpigmented areas. Based on the symptoms and the history of the Patient a generalised phototoxic dermatitis due to rue was diagnosed.

Discussion: More than 200 compounds of *Ruta graveolens* L. have been identified, therefrom 100 essential oils. The isolated components include coumarins, among them the phototoxic furocoumarins (bergapten, xanthotoxin), furoquinoline alkaloids (phototoxic dictamnine and skimmianin) and flavonoids (rutin).

Two forms of furanocoumarin condensation occur in nature. The first forms linear and the second angular furocoumarins. The linear furocoumarins (psoralens) are more phototoxic than the angular (angelicins). The most severe reactions occur with 5-methoxypsoralen (C12H8O4) and 8-methoxypsoralen (C12H8O4). Psoralens have an absorption peak at 300 nm and an action spectrum peak at 335 nm. In an oxygen-independent reaction, UVA excites psoralens to a triplet state that causes covalent binding of the psoralen molecule with nuclear DNA. Monofunctional adducts form between the 4′5′ furan bond or the 3,4 coumarin double bond and the 5,6 bond of a pyrimidine (cytosine or thymidine). When the monoadduct is formed by the 4′5′ furan bond, that product forms bifunctional, interstrand cross-links between pyrimidine bases. Although monoadducts may inhibit DNA synthesis and cell proliferation, stimulate melanogenesis and cause cell death,these effects are greater with cross-link formation. Only the linear psoralens can form bifunctional, interstrand cross-links and these cross-links to keratinocyte DNA are the main chemical change responsible for severe skin damage after UVA. Oxygen-dependent reactions also cause clinical effects. Reactive oxygen species are formed by the interaction of psoralens and oxygen.

The alkaloid dictamnine (C12H9NO2) shows a structure similar to that of the linear furocoumarins and induces monoadducts and to a lesser extent, cross-links DNA in the presence of UVA. In comparison the dictamnine has been proven to be less phototoxic; nevertheless it may play a major role in the elicitation of phytodermatitis because of its abundance in the Rutacae family.

This is the first reported case of a generalized bullous phototoxic dermatitis due to oral consumption of *Ruta graveolens* L. Although, phototoxic dermatitis caused by common rue is extremely rare, dermatologists should consider this scenario in their differential diagnosis and include relative questions while taking the medical history. A provocation test is, however, due to ethical limitations and patient risk not allowed.

P012

A novel method to analyze activation and inhibition (i.e. IgE-dependent) of human mast cells: generation of peripheral CD34+ stem cell-derived mast cells (PSCMCs)

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The use of human mast cells (MC) for high throughput screening (HTS) approaches to identify novel MC inhibitors is hindered by the lack of suitable MC populations and of appropriate detection systems for MC activation and inhibition. Here, we present a novel technique for generating large numbers of well differentiated and functional human MCs from peripheral stem cells (=peripheral stem cell-derived MCs, PSCMCs). Innovative and key features of this technique include the use of stem cell concentrates, which are routinely discarded by blood banks, as the source of CD34+ stem

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cells, cell culture in serum free medium and the addition of LDL as well as selected cytokines. In contrast to established and published protocols, which use CD34+ or CD133+ progenitor cells from full blood, we used a pre-enriched cell population, which yielded up to 10^8 well differentiated human MCs after only 3 weeks. PSCMCs generated by this method reliably show high FCεRI expression, which is up-regulated further by adding LDL, interferons or Interleukin (IL)-1beta. Importantly, the use of this protocol does not result in co-generation of basophils, a problem sometimes seen with other approaches for generating MCs from progenitors. Taken together, this novel protocol for the generation of large numbers of human MCs now allows for HTS of compounds for inhibitory and other effects on human MCs.

P013

Impaired intracellular granule biology: basis of upregulated vigilance mechanisms in children with atopic dermatitis (AD) and/or attention deficit hyperactivity disorder (ADHD)

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Skin, adjacent mucosa and brain are linked by ectodermal origin and function: surveillance of the environment for potential harm and for survival support. Increased skin vigilance is a hallmark of patients with AD/atopy syndrome. This is based in part on reduction of intracellular storage organelles and disturbed granule release mechanisms in several cell systems resulting in an often adequate but not sustained reaction to stimulation. Hypothesis: Since the functional state of sensory organs is not independent of each other, CNS-reactions requiring sustained granule release (from neurons) may be impaired in AD-patients as well. Likewise, ADHD-patients unable to keep up an enduring CNS-response might also show altered peripheral granule biology.

11 children with AD (8 m, 3f), 14 matched ADHD-inpatients with confirmed diagnosis (13 m, 1f), and 8 healthy control individuals (HC) without any atopic or psychiatric background (6 m, 2f) were studied in 3 modules: A) child psychiatry, B) dermato-allergology, C) laboratory. In A, using a neuropsychological computer-based test battery [Psytest 2.2, Herzogenrath, Germany], short term (120 signals / 4 min, 'sprint') and long term (900 signals / 30 min, 'endurance') attentiveness towards visual and acoustic stimuli was tested. ADHD symptoms, emotional and behavioural difficulties as well as personality traits (e.g. novelty seeking) were assessed via questionnaires (CBCL, DISYPS II-ADHD, JTCI). In B, the Erlanger Atopie Score (EAS), SCORAD and a prick test with common aero allergens were performed. In C, total serum IgE, eosinophilic cationic protein (ECP) and differential blood count were determined. After stimulation with ionomycin/PMA, granule release velocity of perforin-containing granules from cytotoxic T lymphocytes (CTL) and of CD63pos secretory lysosomes from basophilis was quantified by flow cytometry.

Module A: 2/11 AD-patients were eliminated from analysis because parents reported a significant number of ADHD symptoms. In the 4 min.- test, AD-children reacted similar to the ADHD-group: significantly slower with more mistakes than HC. In the 30 min.- test, AD-children showed prolonged reaction times as well and slowed down even more over time as compared to HC, but did not make as many errors as ADHD-patients. Module B: 12/14 ADHD-children did not have any AD-symptoms nor any history of AD/atopy. (2/14: rhinoconjunctivitis, positive: skin prick test and family history). ADHD-children showed a white dermographism (13/14) and an EAS of 93 (atopic skin diathesis unclear). 5/11 AD-patients had exacerbated disease (SCORAD > 10, mean EAS 123). Module C: Both, ADHD- and AD-children had elevated (i) IgE levels (7/14, 500 420 kU/l and 5/9, 653 950 kU/l), (ii) eosinophils (9/14, 8.79% and 7/9, 7.76%) and (iii) ECP (8/14, 3130 and 7/9, 3933). In AD- and ADHD-children, perforinpos CTL were significantly reduced (1510% and 126%) as compared to HC (219%, $P < 0.05$). After stimulation with ionomycin/PMA, AD- and ADHD-patients released cytotoxic granules, and upregulated CD63 on the cell surface of basophils, faster and more complete as compared to HC ($P < 0.05$).

Thus, AD/atopy may be interpreted as a consequence of an increased vigilance of skin, immune and nervous system. Reduction and quick release of storage organelles impair sustainability of stimulus induced reactions. Altered granule transport mechanisms in ADHD are first reported here opening a new way to look at ADHD-pathophysiology. Our work may help to understand why atopy is an independent risk factor for ADHD.

P014

Different roles for CD8-positive T cells in IgE-production of patients with extrinsic atopic dermatitis: cell–cell contact dependent inhibition or augmentation in hyper-IgE-patients

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CD8-positive T cells are able to exert regulatory as well as helper T cell function. Evidence in mice and men suggested an inhibitory role in IgE-production. However, other groups have shown convincingly that under certain experimental conditions CD8-positive T lymphocytes can help to augment IgE-production. We now asked whether depletion of this population from ficoll-isolated human PBMC results in elevated or in reduced IgE-levels *ex vivo*.

Therefore, PBMC from 54 individuals (patients with exacerbated intrinsic atopic dermatitis: iAD, $n = 6$, with extrinsic AD/atopy syndrome: eAD, <10 000 kU/l total serum IgE $n = 28$, >10 000 kU/l $n = 14$, and healthy controls: HC $n = 6$) were depleted of CD8-positive T lymphocytes using antibody coated magnetic beads (two purification cycles, MS-columns, Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instruction. 5 × 10 000 cells/1.8 ml round bottom cryovial were incubated in 0.5 ml RPMI supplemented with fetal calf serum for 10d at 37C. Conditions: (i) all PBMC, (ii) PBMC w/o CD8-positive cells, (iii) PBMC CD8-depleted and subsequently reconstituted with CD8-positive cells. Cell free supernatants were collected and stored at -80C until determination of IgE-levels using the ImmunoCap system (low rage level, Phadia, Freiburg).

Under these experimental conditions, CD8-positive T cells were reduced from 51 to 98%. This resulted in elevated IgE-levels as compared to all PBMC in 45% of eAD patients. CD8-reconstitution reversed these phenomenon. IgE-production remained constant in 19% or was reduced in 36% of eAD cases. In iAD or HC, no significant IgE-production was induced by CD8-depletion. In eAD-patients with less or more than 10 000 kU/l serum IgE, CD8-reduction augmented IgE production *ex vivo* in 6/28 (21%) or in 9/14 cases (65%). We then focused on patients where CD8-reduction elevated IgE. Whereas in mice cell-cell contact is essential for this CD8-mediated effect, this was unclear in humans. Therefore, in 6 eAD-patients, the experiment was modified: after 2 CD8-depletion cycles, cells were seeded as described above, however, now in 24 well flat bottom plates (Corning, 6.5 mm transwell inserts, 0.4 m pore size). An additional condition was introcuced: (iv) CD8-depleted PBMC (lower transwell chamber) and CD8-positive cells (upper chamber).

According to our data, eAD-patients may be divided in three groups: (i) In the majority, CD8-reduction elevated IgE *ex vivo*. CD8-reconstitution with cell-cell contact abandoned this elevation which confirms and extends substantially earlier findings. In contrast, CD8-reconstitution in the transwell system, i.e. without cell-cell contact, did not influence IgE-production in the same way, again extending our previous data. Thus, the majority of IgE-controlling mechanisms *ex vivo* by CD8 positive T cells seems to be dependent, at least in part, of T-B cell contact. (ii) In a minority of cases (19% of eAD), CD8-depletion had no effect on IgE-production suggesting that these cells do not play a role in IgE-control in this group. (iii) In a substantial group of eAD-patients, CD8-reduction reduced IgE-levels significantly. This was the case in most of eAD-patients with very high total serum IgE-levels. Thus, in these individuals, the inhibitory role of CD8-positive T cells seems to be supplanted by a T helper function which now facilitates IgE-production. If they can be 'switched back' to their original regulatory function will have to be determined in the future.

P015

Impact of specific immunotherapy with birch pollen extracts on basophils

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Background: Basophils are well known effector cells in IgE-mediated allergies, characterized by the secretion of histamine, leukotrienes and prostaglandins after IgE bound to their high affinity IgE receptor FcεRI is cross-linked by allergen. However, basophils can also exert important functions in promoting and modulating Th < sub > 2 < /sub>-mediated immune responses, for example by secretion of IL-4 and IL-13. Since specific immunotherapy (SIT) results in decreased activity of allergen-specific Th < sub > 2 < /sub>-cells, we assessed the influence of SIT on basophils of birch pollen (BP) allergic patients undergoing or having finished SIT.

Methods: Allergen specific reactivity of basophils was determined during BP season in BP allergic patients currently treated by or having stopped SIT and compared with BP allergic patients nave to SIT (all $n = 7$) and healthy controls (HC; $n = 8$). After stimulation with different concentrations of BP extract or the major BP allergen, Bet v 1, the expression levels of the activation marker CD 63 or the immunoglobulin receptors FcεRI and FcγRII (low affinity IgG receptor) were quantified by flow cytometry analysis.

Results: While SIT resulted in a pronounced reduction of clinical symptoms in BP allergic patients, neither allergen-specific CD 63 nor FcεRI expression levels, which correlated with individual amounts of total IgE serum concentration, differed between the four groups. However, all three groups of BP allergic patients showed decreased basophil FcγRII expression compared to HC. Interestingly, this difference was abolished by SIT resulting in comparative levels after finishing SIT.

Conclusion: Our results suggest that SIT is accompanied by an increase of FcγRII expression on basophils which might be of importance in achieving allergen tolerance.

Cellular Biology

P016

Signal transduction in response to mechanical stretch – impact of desmosomal structures and the keratin filament

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Mechanical stress is an ubiquitous challenge of human cells with impact on cell physiology. Previous studies have shown that stretching of skin cells promotes signaling cascades involved in proliferation and tissue enlargement. The present study is dedicated to learn more about cellular structures contributing to perception and signal transmission of cell stretch. In particular, we hypothesized that desmosmal contacts and the adjacent keratin filament build an intercellular matrix providing information about the mechanical load. It was found that the omission of calcium from the medium, a necessary cofactor for desmosomal cadherins, inhibited stretch mediated activation of PKB/Akt and p44/42. The relevance of desmosomes in this context was further examined by experiments using a desmoglein 3 blocking antibody (AK23) showing modest impact on protein activation. Moreover, disruption of the keratin filament by sodium orthovanadate completely abrogates PKB/Akt and p44/42 activation in response to stretch. These findings give a first hint for a contribution of desmosomes and keratins in mechanosensing.

P017

Characterization of the protease inhibitor SPINK7 in human skin

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Kallikrein-related peptidases (KLKs) are involved in the desquamation process and induce epidermal inflammation by different mechanisms. Their activity is tightly regulated by protease inhibitors, especially of the Kazal-type inhibitors SPINK1, SPINK5, SPINK6 and SPINK9. Their genes are encoded on a cluster on chromosome 5. Another gene in this chromosomal region is SPINK7. Since little is known about this gene and its product in skin biology, we were interested in exploring SPINK7 expression and function in human skin.

Spink7 mRNA expression was detected in some but not all skin biopsies investigated. Focal SPINK7 expression was detected in the human epidermis and in the stratum granulosum of a skin three dimensional model by immunohistochemistry. In cultured keratinocytes. spink7 mRNA expression was induced by IL17alpha together with TNFalpha but not other cytokines investigated. Recombinant SPINK7 exhibited no inhibition of KLK5, KLK7 and KLK8. In summary, SPINK7 was detected for the first time in human skin. In contrast to SPINK5, SPINK6 and SPINK9, its expression is regulated upon specific cytokines and it is no inhibitor of epidermis-derived KLKs. Further investigations are needed to clarify the role of SPINK7 in human skin.

P018 (O20)

Suppression of neutrophil-mediated tissue damage – a novel skill of mesenchymal stem cells

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Neutrophils, if overactivated, can cause severe tissue damage in infectious and non-infectious neutrophilic dermatoses such as pyoderma gangrenosum, immune-complex (IC) mediated vasculitis and chronic venous leg ulcers, disorders with remarkable morbidity which often poorly respond to conventional therapies. Mesenchymal stem cells (MSCs) exert beneficial effects on chronic wounds by dampening unrestrained inflammation of both adaptive and innate immune cells mainly suppressing macrophages. Though of prime clinical importance the effects of MSCs on neutrophils so far have not been studied in sufficient detail. Therefore, we set out to investigate the effects of human adipose tissue derived MSCs on neutrophil activation. MSCs significantly reduced the respiratory burst with unrestrained release of reactive oxygen species (ROS) of co-cultured PMA-activated human and murine neutrophils. In addition, MSCs suppressed the release of active neutrophilic enzymes involved in tissue damage including myeloperoxidase, elastase and gelatinase (MMP-9) from co-cultured murine neutrophils. Interestingly, as shown by confocal microscopy, MSCs actively phagocytosed apoptotic neutrophils thus preventing the extracellular spillage of tissue damaging proteolytic enzymes and ROS. In this regard also the formation of neutrophil extracellular traps (NETs) indicative of neutrophil break down and tissue damage was substantially inhibited by MSCs. The suppressive effects of MSCs on activated neutrophils were further confirmed *in vivo* in murine models of PMA-induced neutrophilic skin inflammation and IC-mediated neutrophil-dependent reverse passive Arthus reaction. Intradermally injected MSCs resulted in a 40% reduction of ROS release in PMA-induced skin

inflammation as detected by *in vivo* imaging with a ROS-sensitive chemiluminescent probe. Similarly, MSC-injection reduced oedema and haemorrhage formation, and thus the severity of IC-induced vasculitis in the skin by 50%. Preliminary data suggest that MSCs are able to adaptively build up an antioxidant shield via upregulation of superoxide dismutases SOD2 and SOD3, and thus effectively interrupt the vicious cycle of ROS-induced proteolytic and oxidative tissue damage caused by overactivated neutrophils. In summary, MSCs – responding to the need for self- and tissue protection – have developed a variety of strategies to reduce neutrophil-dependent cytotoxicity, and, if therapeutically employed, may hold substantial promise to counteract unrestrained tissue damage in conditions caused by overactivated neutrophils.

P019

Fetal human keratinocytes produce high amounts of antimicrobial peptides: involvement of DNA-methylation processes

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Antimicrobial peptides (AMP) are an important part of the innate immune system and play a crucial role in the skinEUR(TM)s defense against invading micro-organisms. Whereas AMP have been extensively studied in adult skin little is known about their expression and regulation in the developing skin. Here we investigate the expression and regulation of AMP in fetal, neonatal and adult KC *in vitro*.

We found that the constitutive expression of human beta defensin-2 (HBD-2), members of the S100 protein family (S100A7, S100A8 and S100A9) and cathelicidin were significantly higher in cultured KC from fetal skin than in KC derived from neonatal or adult skin and this difference was maintained even after several passages *in vitro*. By contrast the capacity to further increase AMP-production using inflammatory triggers such as IL-1 or TLR-ligands was comparable between pre- and postnatal KC. Further analysis of skin equivalents (SE) generated with KC from different age groups revealed a strong constitutive expression of S100 proteins in fetal but not in neonatal and adult SE. In addition, we found higher expression of the histone demethylase JMJD3 in prenatal KC, which significantly correlated with the expression of S100A7, S100A8 and cathelicidin.

In summary, we could show that fetal KC constitutively express high amounts of several AMP. The correlation with JMJD3 expression might suggest that DNA methylation processes are involved in the regulation of AMP in pre- and postnatal KC. Since in prenatal skin the adaptive immune system is not yet fully functional, the high AMP expression might therefore represent an important defense strategy of the unborn.

P020

Pigmentation survey: from monolayer to pigmented organotypic tissue cultured skin equivalents

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Skin color is a very prominent aspect of the human complexion. Even minor defects lead to stigmatization. Apart from pigmentation disorders, changing the skin color to fit certain beauty ideals is common. In Asian and African countries skin bleaching is popular, whereas in European countries moderate skin darkening mostly achieved either by sun tanning or tanning lotions is prevalent. Analyzing the influence of de-pigmenting or hyper-pigmenting agents is mostly performed on monolayer cultures. Aim of this study was the development of an organotypic tissue cultured skin equivalent (TCSE), inducing hyper- und hypopigmentation and evaluating different analytical tools.

To create the TCSE primary fibroblasts and epidermal cells were enzymatically isolated from foreskin. After *ex vivo* expansion the cells were seeded into scaffolds and cultivated according to common protocols. Characteristically fibroblasts inhabited the matrix and keratinocytes formed a stratified epidermis containing stratum basale, -spinosum, -granulosum and -corneum at the air-liquid-interface. Melanocytes were exclusively found in the stratum basale whereas their dendrites were found in all vital epidermal layers. Hyperpigmentation was induced by 40M forskolin and 250M kojic acid was applied as hypopigmenting agent. To evaluate the effect of forskolin and kojic acid monolayer cultures of melanocytes from skin type III or skin type VI were treated with these two agents for different periods of time. Monolayers were harvested and melanin was extracted and quantified by photometer. Pigmentation of epidermal equivalents and of TCSEs was induced or respectively inhibited by the same treatment regime. Quantification could be obtained either invasively by melanin extraction or noninvasively by the pigment station SP99. Melanin extraction showed that kojic acid reduced the melanin concentrations in comparison to the controls by 25% in monolayer and by 25% to 41% in epidermal equivalents or TCSEs. The macroscopic observable hyperpigmentation by treatment with forskolin was proven due to a 20% to 40% higher melanin concentration in comparison to the controls. Melanin concentration measured with the pigment station showed equal effects. The melanin content was 35% lower than that of the controls after treatment with kojic acid and 40% to 60% higher than the controls after treatment with forskolin.

The present study demonstrates the comparability of the developed organotypic tisse cultured skin equivalent to healthy human skin and the versatility of its utilizations.

P021

Biodentine® a dentine substitute reduces cell viability as well as collagen type I synthesis in pulpa fibroblasts

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Many dermatologic diseases also affect the oral mucosa therefore synergistic investigations concerning the influence of externally added compounds to cells of the oral cavity are relevant for dentistry as well as dermatology.

The newly developed tricalcium silicate based cement Biodentine® is used as dentine substitute or capping agent in dentistry. It is postulated that Biodentine® is biocompatible and bioactive. Aim of this study was to investigate the influence of Biodentine® on primary pulpa fibroblasts isolated from freshly extracted wisdom teeth. Biodentine® was eluated according to the manufacturer's instructions. The Biodentine® paste was spread on a silicon molding tool to obtain Biodentine® discs with a diameter of 5.1 mm. One Biodentine® disc to five Biodentine® discs were incubated in culture media. The media were collected and replaced with fresh medium every 24 h for 5 days. Pulpa fibroblasts were treated with these eluates for 24 h to 48 h. Proliferation, cell viability, toxicity and collagen synthesis were monitored.

It could be shown that none of the Biodentine® eluates was toxic and therefore cell integrity was maintained. Proliferation was also not influenced by the eluates whereas cell viability was diversely regulated. When applying one Biodentine® disc cell viability was enhanced whereas a distinct cell viability reduction for eluates collected after the first 24 h containing five Biodentine® discs was observed. Furthermore concentration dependent effects on cell viability could be seen when applying the day 1 eluates containing different amounts of Biodentine® discs. To evaluate the effect of Bidentine® on collagen type I synthesis the concentration of the N-terminal domain of pro-collagen type I (P1NP) was quantified. The P1NP concentration of all pulpa fibroblast cultures treated with the eluates of five Biodentine® discs of day 1 was reduced to 10% of the P1NP concentration of the untreated control. The P1NP concentrations of the cultures treated with the Biodentine® eluates of day 2 to day 5 were reduced to 60% to 80% of the control level.

In summary, the present findings indicate that Biodentine® influences cell viability and collagen synthesis. Further studies concerning the mechanisms of collagen synthesis regulation in dependence of Biodentine® have to be conducted.

P022

Combination of low doses of curcumin with UVA or VIS induces apoptosis in head and neck squamous cell carcinoma cells

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Many dermatologic diseases also affect the oral mucosa therefore synergistic investigations concerning the influence of externally added compounds to cells of the oral cavity are relevant for dentistry as well as dermatology.

Curcumin a well-known dietary pigment from the plant Curcuma longa is known for its ability to inhibit cell proliferation and induce apoptosis in different cell lines. Earlier studies from our laboratory showed the effect of low curcumin concentrations (0.2–1 g/ml) in combination with UVA or visible light (VIS) on A431 and different melanoma cell lines.

In this study we focused on head and neck squamous cell carcinoma cells (HN). Curcumin was used at different concentrations ranging from 0.01 to 0.8 g/ml.

Curcumin alone in this concentration range neither influenced proliferation, nor cell integrity nor apoptosis. It could be shown that curcumin in combination with 1J/cm^2 UVA at concentrations of 0.4 g/ml clearly reduced cell proliferation. Applying higher curcumin concentrations than 0.6 g/ml in combination with UVA or VIS induced membrane damage, proven by monitoring the LDH concentration in cell free supernatants. When irradiating HN cells for 5 min with VIS a curcumin concentration dependent proliferation reduction could already be observed for cultures treated with 0.2 g/ml curcumin. Monitoring apoptosis revealed a clear concentration dependent increase of apoptosis in UVA as well as in VIS treated cell cultures when administering curcumin concentrations from 0.05 g/ml.

In summary, the present findings substantiated the usefulness of the combination of curcumin and light as a new therapeutic concept to increase the efficacy of curcumin in the treatment of cancer of oral mucosa.

P023

TRAF2 inhibits death receptor-induced apoptosis and necroptosis in keratinocytes

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The subgroup of death receptors within the TNF receptor family can induce apoptosis, but also necroptosis and activation of NFB and MAPKs. The outcome of receptor triggering depends on the interplay of inducible receptor-associated and cytosolic protein signalling complexes. The relevance and functions of the adaptor protein TRAF2 and the TRAF2-associated E3-ligases cIAP1 and cIAP2 for signal transduction of the death receptor TNF receptor-1 (TNFR1) and CD95 are well established [Geserick et al, JCB 2009]. Whether TRAF2 also plays a role in signalling by death receptors CD95 and TNF-related apoptosis inducing ligand (TRAIL)-receptor-1 (TRAILR1) and TRAILR2 is poorly understood.

When we repressed TRAF2 expression by siRNA in HaCaT keratinocytes and analyzed TRAIL- and CD95L-induced cell death, we observed a significant sensitization towards TRAIL – and CD95L – induced apoptosis as compared to control cells. Most interestingly, while cell death induction in control cells was fully blocked by the pan-caspase inhibitor (zVAD-fmk), HaCaT cells challenged with TRAIL or CD95L after TRAF2 knockdown were only partly rescued. The kinases RIPK1 and RIPK3 are thought to be crucial for caspase-independent necroptosis and the formation and regulation of an intracellular protein complex mediating death receptor-induced necroptosis. These complexes have been termed ripoptosome, TNF complex II, or the necrosome. In line with an involvement of such intracellular death complexes, TRAF2-repressed HaCaT were almost fully protected in the presence of zVAD-fmk combined with either the RIP kinase-1 (RIPK1) inhibitor necrostatin-1 and/or with the mixed lineage kinase domain-like (MLKL) inhibitor necrosulfonamide, two inhibitors of necroptotic cell death. Primary keratinocytes, which are relatively resistant towards death receptor-mediated cell death could also be sensitized for TRAIL – or CD95L -induced cell death, especially towards caspase-independent necroptosis.

To better understand the relation of TRAF2 to RIPK1- and RIPK3-dependent necroptosis and RIPK3-independent caspase-mediated apoptosis, we repeated our experiments in HeLa cells that lack endogenous RIP3. Control HeLa cells were markedly sensitized to TRAIL-induced caspase-dependent cell death by TRAF2 knockdown. In RIPK3 expressing HeLa cells, however, knockdown of TRAF2 strongly sensitized towards necroptosis, in line with data in HaCaT keratinocytes, which express high levels of endogenous RIPK3.

Taken together, TRAF2 is an important negative regulator of death receptor-induced apoptosis and necroptosis. This depends on the activation state of caspases and on cellular levels of RIPK3. Altering/diminishing cytoplasmic protein levels of TRAF2 could thus restore sensitivity for death receptor-induced cell death in resistant cells and thus might have potential in tumor therapy.

P024

3-Dimensional poly(lactide-co-glycolide) scaffolds coated with modified extracellular matrices are functional growing substrates for dermal fibroblasts

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3D biomaterials require the application of a structural element serving as a scaffold and a functional element regulating cell physiological processes like adhesion, proliferation or differentiation. The application of native or modified extracellular matrix (ECM) components can functionalize the scaffolds for cells. We used artificial ECM (aECM) consisting of the structural protein collagen I (coll) and hyaluronan (HA) or appropriate chemically sulfated HA derivatives. Sulfate groups are expected to concentrate and improve bioactivity of growth factors near the cells. Our previous work with 2D-aECM had shown that initial adhesion and cell proliferation of dermal fibroblasts (dFb) progressively increased in a sulfate-dependent manner. In contrast, synthesis of ECM components coll and HA and the matrix metallo-proteinase-1 (MMP-1) was decreased on high-sulfated aECM on mRNA and protein level (van der Smissen et al., Biomaterials, 2011).

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Here, cultures of dermal fibroblasts in porous poly(lactide-co-glycolide) (PLGA) based scaffolds coated with coll and HA or sulfated HA were used. We found a dense colonisation of scaffolds by dFb within 7d in the pores while cells on the surface were organized in a multilayer. Fb actively migrated into the PLGA-scaffolds from surrounding collagen gels and responded to TGFβ1-mediated induction of gene expression thus proving the biocompatibility of the scaffold. Histological stainings indicate that cells proliferate and deposit collagens and HA in the scaffolds.
Next, the scaffolds were adsorptively coated with aECM. Fibroblasts proliferate in these scaffolds. However, sulfation of HA in the aECM did not alter the cell proliferation. The expression of collagen I (α1) mRNA decreased with time while MMP-1 was expressed at higher levels suggesting the induction of matrix remodelling processes. Addition of TGFβ1 improved cell proliferation in all scaffolds. The sulfation of GAG however, decreased the effects of TGFβ1 concerning fibroblast differentiation and matrix synthesis as known from previous investigations in 2D systems (van der Smissen et al., Acta Biomaterialia 2013). Taken together, coatings of aECM are suitable for growing dFb and support the synthesis and remodelling of ECM by dFb in 3D systems. The modification of the HA component can alter the effects of TGFβ1 and putatively of other factors.

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P025

Reversal of murine epidermal atrophy by topical modulation of calcium signaling

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Cytosolic Ca^{2+} signals are performed by Ca^{2+} releases from the endoplasmic reticulum and Ca^{2+} influx from the extracellular medium. Releases rely on the refilling of the intracellular Ca^{2+} stores by the Ca^{2+} influx 'Store Operated Ca^{2+} Entry' (SOCE) via the channel Orai1. Here we show that Orai1 expression, SOCE amplitude and epidermal proliferation are decreased in the epidermis of patients with dermatoporosis when compared to aged non-atrophic skin. Epidermal atrophy was induced in mice by the inhibition of Orai1 with small interfering RNA and the topical application of a SOCE blocker, BTP2. The inhibition of Orai1 impaired the HB-EGF-induced Ca^{2+} influxes and fully prevented the mitogen effect of HB-EGF in human primary keratinocytes (HPK). Importantly, epidermal proliferation correlated with Orai1 expression in mice. Conversely, the topical application of an Orai1-activator, the benzohydroquinone (BHQ), increased epidermal thickness and proliferation in mice while the pro-proliferative effect of BHQwas prevented by the inhibition of Orai1 in HPK. Finally, the topical application of BHQ reversed the epidermal atrophy induced by corticosteroids in mice. The topical modulation of Ca^{2+} signals may thus be a new and promising therapeutic strategy in dermatology.

P026 (O18)

Role of NADH-dehydrogenase subunit 2 mutation in fibroblast ageing

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Mitochondrial production of reactive oxygen species (ROS) is suggested to significantly contribute to organismal ageing, but the specific role of individual mitochondrial pathways is still unclear. Presumably, a dysfunction of parts of the mitochondrial respiratory chain leads to enhanced ROS production, DNA damage, cellular senescence and ageing. The characterization of relevant respiratory chain molecules and pathways activated under these conditions could provide deeper insights into the process of ageing.
In the present study, different conplastic mouse strains were used harboring mutations in mitochondrial genes encoding respiratory chain protein complexes I-V and uncoupling protein 2 (UCP2). These mice were analyzed at different time points for expression and secretion of age-related markers (3, 6, and 12 months). For this purpose, isolated primary skin fibroblasts were either left untreated or were exposed to cellular stresses like doxorubicin and hydrogen peroxide. Under cellular stress conditions ROS, ATP, age-related cytokines and cellular proliferation were measured and compared to basal level.
The mouse strain C57BL/6J-mtALR/LTJ with a single nucleotide exchange (nt4738A) in the NADH dehydrogenase subunit 2 gene in complex I showed decreased ROS and enhanced ATP basal levels compared to the control strain C57BL/6J-mtAKR/J, in 12- month-old mice. The mutated strain showed an enhanced proliferation rate compared to the control strain detected by BrdU incorporation. Furthermore, IL-6 and IL-8 levels in C57BL/6J-mtALR/LTJ mice increased 4 and 8 days after doxorubicin treatment, but the basal secretion level of these cytokines was lower compared to the control strain. Immunoblots showed that expression of age-related marker H3K9me3 and expression and activation of stress signaling protein IκBα were delayed in C57BL/6J-mtALR/LTJ mice after doxorubicin treatment.
These results demonstrate obvious differences in baseline and stress-induced ageing markers and stress signaling between mutant and control mice. The reaction patterns of skin fibroblasts of C57BL/6J-mtAKR/J mice may be interpreted to exhibit an increased resistance to oxidative and cellular stresses with the consequence of delayed ageing. Experiments regarding lifespan of the different mouse strains are ongoing. However, it has been shown in humans, that a single nucleotide polymorphism (C5178A) in the NADH dehydrogenase subunit 2 gene is associated with longevity in a Japanese population. Taken together, in the present report we identified a mitochondrial gene that may be age-protective in mice and humans using well-defined signaling and secretory pathways.

P027

Biocompatibility of aminocellulose in a co-culture model of human HaCaT keratinocytes and *Candida albicans*

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Introduction: Naturally occurring macromolecules like cellulose can be altered by chemical modification. Aminocelluloses are produced by directed functionalization of cellulose with amino groups. It is thought that aminocelluloses possess significant antimicrobial effects due to their positive charged amino groups. Hence, aminocelluloses might recommend themselves for a broad spectrum of applications, e.g. as antimicrobial wound dressings. However, good cell compatibility is of crucial importance for medical uses. In this study, the biocompatibility of different aminocelluloses was tested using an *in vitro* co-culture model of human HaCaT keratinocytes and *Candida albicans*. Here, aminocelluloses tested were functionalized with ethylendiamine (EDA) and differ in the degree of substitution of the amino groups bonded to cellulose backbone (DSamin).
Methods: Antimicrobial activity of the EDA-aminocelluloses with DSamin = 0.33 (EDA0.33) or DSamin = 0.54 (EDA0.54) against *Candida albicans* DSM 1386 was determined by microplate laser nephelometry (MLN) and by measurement of the ATP content (BacTiter-Glo(TM), Promega). In addition, effects on human HaCaT keratinocyte were analyzed by measuring the cellular ATP content (ATPLite(TM)-M, PerkinElmer) and the total protein amount (BC Assay Protein Quantitation Kit, Interchim). Furthermore, biocompatibility of both aminocelluloses was tested using an *in vitro* co-culture model of HaCaT keratinocytes and *C. albicans*. Here, the quantification of *C. albicans* was carried out by qPCR in a Rotor-Gene® Q (Qiagen) with the primer 5′-TGAAGAACGCA-GCGAAATGC-3′ and 5′-GCAAACCCAAGTCGTATTGC-3′ (Eurofine).
Results: Both aminocelluloses demonstrated good cell compatibility and a high antimicrobial activity. By means of the results of the individual tests, half maximal lethal (LC50) and inhibitory concentrations (IC50) were determined in regard to cell compatibility and antimicrobial activity. Although, EDA0.33 (LC50 = 3941 g/mL) showed a better cell compatibility than EDA0.54 (LC50 = 865 g/mL). The antimicrobial activity against *C. albicans* in turn was higher for EDA0.54 (IC50 = 427 g/mL) compared to EDA0.33 (IC50 = 611 g/mL). Compared to the individual tests, higher concentrations of both aminocelluloses were necessary to inhibit *C. albicans* in the co-culture model.
Conclusions: In general, the tested aminocelluloses with different DSamin exhibited a good biocompatibility against human HaCaT keratinocytes with a high antimicrobial activity against *C. albicans*. The results of this study indicate that the DSamin of aminocelluloses seems crucial for their biocompatibility.

P028

Hemocompatibility evaluation of functional biopolymers *in vitro*

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Objective: Functional biomacromelecules (FBM) offer application opportunities ranging from technical to medical uses, including implant coatings, wound dressings and antimicrobial surfaces. Especially for medical applications, the utilization safety, bio- and hemocompatibility are important. Hence, in accordance with the ISO 10 993–4, different assays were used to evaluate the *in vitro* hemocompatibility of four FBM differing in their degree of substitution with amino groups (DSamin: 0.5–0.9). Markers for coagulation (thrombin generation, activated partial thromboplastin time (aPTT), prothrombin time (PT), blood clotting, thrombocyte activation) and compatibility (hemolysis) were measured in human whole blood, human platelet rich plasma and human pooled plasma, respectively.
Methods: Aqueous solutions (1% w/V) and appropriate dilutions in aqua dest. of the four FBM (DSamin: 0.5; 0.7; 0.8; 0.9) were used in this study. For determination of the plasmatic thrombin generation *in vitro* the Technothrombin® Thrombin Generation Assay (TGA) from Haemochrom Diagnostica GmbH was used. PT and aPTT were measured in human pooled plasma with coagulation analyzer MC1 (Greiner Biochemica GmbH). Blood coagulation was analyzed by incubation of FBM with human blood. The amount of activated platelets in platelet rich plasma was measured by flow cytometry analysis in the BD FACSCanto (BD Biosciences). FBM-dependent hemolysis of human erythrocytes was measured photometrically using the SPECTROstar Omega (BMG Labtech GmbH).
Results: Thrombin is the central serine protease in the plasmatic coagulation cascade. Time response of thrombin generation in recalcified pooled human citrate plasma was assessed with the TGA. Concentration- and DS-dependent inhibition of thrombin generation was detected for all four FBM. Correspondingly, extension of lag time and time to peak of the thrombin generation-time-curve were observed. Plasmatic, secondary hemostasis can be activated by the extrinsic and intrinsic pathway. FBM inhibited both pathways in a concentration- and DS-dependent manner, as shown by prolongation of PT and aPTT. Moreover, effects of FBM depending on both, concentration and DS, on blood clotting and hemolysis were found. Human platelets were activated over 80% by concentrations >0.5 g/mL by all FBM tested, as shown in flow cytometry assays.
Conclusion: Influences of FBM on the complex hemostatic pathways are not yet fully understood. However, it is known that polycationic polymers interact with negatively charged proteins or phospholipid membranes. Therefore, hemocompatibility evaluation is important for substances contemplated for biomedical applications. In this study, a correlation between the degree of substitution and the inhibitory effects of FBMs was demonstrated *in vitro*, e.g. higher substitution affected coagulation more. Thrombin generation (TGA), blood clotting and both extrinsic (PT) and intrinsic activation (aPTT) pathways of coagulation were repressed by the FBM tested in a DS- and concentration-dependent manner. FBM with lower degree of substitution exhibited a higher hemocompatibility *in vitro*, suggesting possible biomedical application. However, flow cytometric analysis of platelet activation revealed high activation potentials of all FBM tested. Hence, despite inhibition of coagulation in different *in vitro* assays, caution is essential for *in vivo* biomedical uses as platelet activation can be a source for thromboembolic events. In conclusion, the use of different *in vitro* assays demonstrates the complexity of interactions of FBMs with the hemostatic system.

P029

Integrin-linked kinase regulates differentiation and carcinogenesis in the skin

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Integrin-linked kinase (ILK) is a pseudokinase and an important adaptor protein that links integrins to the actin cytoskeleton. Through its impact on cellular force generation, actin remodeling and focal adhesion dynamics, it regulates central processes such as cell migration and matrix deposition. The epidermal deletion of ILK in mice leads to severe defects in skin homeostasis marked by progressive hair loss, epidermal multilayering and skin blistering.
We aimed to understand the molecular mechanisms of these phenotypes and therefore analyzed pathways that regulate keratinocyte fate. These analyses showed that ILK-deficient cells display a less differentiated phenotype characterized by a gene expression signature resembling activated stem cells. This observation prompted us to analyze tumor formation in these mice.
We therefore subjected mice to the two-stage skin carcinogenesis protocol, where we observed an increase in tumor incidence and multiplicity in the ILK-deficient mice. We hypothesize that loss of ILK might lead to an activation of progenitors that subsequently fail to differentiate. Accumulation of these activated progenitors in the epidermis would subsequently predispose the tissue to transformation and subsequent papilloma formation.

P030

In situ aged human dermal fibroblasts exhibit secretome changes associated with spontaneous senescence

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Aging is a multi-factorial process, in which endogenous changes and exogenous factors are likely to contribute equally. With regard to skin aging many mechanisms converge on the dermal stroma consisting mainly of dermal fibroblasts and their surrounding matrix. Since the dermis, in contrast to the epidermis, is a mostly post-mitotic compartment, ageing-associated alterations within the cells are

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likely to accumulate with age and cellular function adapts/maladapts accordingly. Here we report on a systematic study of key features of cellular senescence and the cellular secretome in primary fibroblasts derived from breast reduction surgery from human donors of three age goups: 20–29, 40–49 and over 60 years of age.

In order to detect alterations related to donor age and minimize confounding influences, we (i) concentrated on functional alterations that are conserved or revealed in primary culture of the cells. (ii) All cells were studied at the same early stage of population doubling, where significant telomere shortening and corresponding features of replicative senescence could be excluded. (iii) The study was restricted to dermal fibroblasts from female donors, thus excluding the gender influence on skin ageing. (iv) All cells studied were isolated from the same skin area (bottom side of the female breast), thereby minimizing variances due to body location or different exposure to external stimuli.

We find that *in situ*-ageing of dermal fibroblasts encompasses cellular senescence, but the senescent phenotype does not recapitulate all features reported from fibroblasts subjected to stress-induced or replicative senescence in culture. Most notably, the incidence of DNA-SCARS increased with donor age. Moreover, we observed numerous, age-related changes in the secretome of the cells that are in principle consistent with the acquirement of a senescence associated secretory phenotype (SASP), even though the composition of secreted factors was strikingly different from the one reported in association with cellular senescence induced *in vitro*. This discrepancy possibly indicates that there exist different types of SASP that are specifically associated with different triggers and phenotypic variations of cellular senescence. The ageing-related onset of features of cellular senescence observed in this study was spontaneous, it occurred without an additional external trigger such as irradiation. Therefore it is plausible to assume that the cellular senescence program was triggered by intrinsic, age-related dysfunctions.

P031 (O34)

Aging impacts the niche and key regulatory signaling pathways in skin stem cells

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Aging not only causes visible changes in the mechanical properties of the skin revealed by loose, wrinkled and stiff skin, but also results in loss of regenerative potential shown by impaired wound healing capacities. However, it is not known whether the changes in the mechanical properties of the skin directly impact the regenerative potential of stem cells (SC). We aimed at understanding the role of mechanotransduction in maintaining skin homeostasis in mice during aging. Our results show that aged mice (2 years) show increased matrix stiffness and increased heterogeneity in the mechanical properties of the dermal SC niche, as measured at nanoscale by atomic force microscopy. This was concomitant with higher content of trifunctional collagen crosslinks and a thicker basement membrane as compared to their young counterparts (6 months). Interestingly, these aged mice showed reduced numbers of SCs at the bulge, a well characterized, bona fide multipotent stem cell niche in the hair follicle of the skin. Molecular analyses of signaling pathways regulating SC fate in skin revealed that key signaling pathways are differentially regulated in these SCs. Interestingly, *in vitro* studies further revealed that some of these pathways were mechanoresponsive. We therefore hypothesize that changes in the microenvironment/niche of the skin during aging may lead to changes in the signaling pathways required in maintaining SC quiescence, which might subsequently lead to a loss of these stem cells from their niche via differentiation towards a progenitor fate.

P032

Mammalian polarity proteins in skin homeostasis

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The establishment and maintenance of cell polarization is crucial for development and tissue homeostasis. The three evolutionarily conserved polarity complexes Scribble, Crumbs and Partitioning defective (Par) play an important role in cell polarization. The ubiquitously expressed Par complex consists of Par3, atypical protein kinase C (aPKC) and Par6. In a Ras-driven skin tumor model, we recently demonstrated that epidermal deletion of Par3 reduces the incidence and growth of papillomas whereas it predisposes mice to the formation of keratoacanthomas (Iden et al., 2012). These studies revealed a dual function of Par3 both as tumor suppressor and tumor promoter, respectively.

Keratinocytes undergo considerable morphological changes during their differentiation thus posing the question whether the apical polarity proteins, which in simple epithelia are required for the establishment of apico-basal polarity, also contribute to this process. To assess a potential role of Par3 in the homeostasis of a stratified epithelium, we investigate mice with epidermal Par3 deletion (Par3 eKO) at different developmental and adult stages. Loss of Par3 results in progressive hair loss and disturbed homeostasis of the epidermis and its appendages. Par3 eKO mice exhibit increased Keratin-1/-10 levels, delayed formation of tight junctions (TJs) with reduced levels of TJs proteins such as ZO-1 and claudin-1. The loss of Par3 also results in temporary increased epidermal thickness, progressive decline of hair follicle stem cells and enlarged sebaceous glands in aging mice. These results indicate that Par3 regulates the overall cell fate by controlling the balance of stem cell maintenance versus differentiation in the epidermis and hair follicle. We currently address the role of Par3 in tissue regeneration processes including wound healing, and the regulation of Par3 in epidermal stress signalling pathways.

Together, by investigating a mammalian self-renewing stratified epithelium, which is exposed to environmental and cellular stress, and by manipulating the function of conserved polarity regulators in this system, we will be able to unravel mechanisms through which the Par complex contributes to skin homeostasis and regeneration. Since many processes fueling epidermal homeostasis occur to some extend in other epithelia, our studies will also have implications in understanding the maintenance and regeneration of other tissues.

P033 (O05)

Thy-1 (CD 90) – a tumour suppressor on dermal fibroblasts?

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Thy-1 (CD 90) has been described as a cell-cell adhesion molecule mediating the interaction of myeloid cells and melanoma cells to activated endothelial cells. In contrast to endothelial cells, Thy-1 is constitutively expressed on fibroblasts. Differences in proliferation, migration as well as cytokine and growth factor responses investigated in Thy-1+/+ and Thy-1−/− subpopulations of pulmonary fibroblasts implicate further functions of Thy-1 independent of its role as a cell adhesion molecule. But there are no data available about the functional role of Thy-1 on fibroblasts. In the present study, we investigated the effect of Thy-1 expression on dermal fibroblasts with respect to cell proliferation, apoptosis, senescence and differentiation using fibroblasts from Thy-1-deficient mice, wild type (wt) mice and fibroblasts from mice containing loxP-flanked Thy-1 genes and a tamoxifen inducible Cre recombinase under the control of the collagen type I promoter (Col1α2-Cre). We demonstrate that a lack of Thy-1 resulted in a significantly higher proliferation rate compared to wt fibroblasts. Furthermore Thy-1−/− fibroblasts showed less apoptosis and cellular senescence. Moreover, more Thy-1−/− fibroblasts were found in G2/M phase than wt fibroblasts. In contrast, Thy-1+/+ fibroblasts displayed a more differentiated phenotype reflected by an enhanced ability to contract free-floating collagen lattices as well as an increased expression of the myofibroblast marker alpha-smooth muscle actin (α-SMA). The increased proliferation of Thy-1−/− fibroblasts was completely abolished by seeding Thy-1−/− fibroblasts on immobilized recombinant Thy-1. It is known that the interaction of Thy-1 with several integrins is mediated by its RGD-like integrin-binding sequence (RLD). Consistently, culture of Thy-1−/− fibroblasts on recombinant Thy-1 with a mutation in the integrin-binding motif (RLD to RLE) affected proliferation of Thy-1−/− fibroblasts significantly less than wt Thy-1. Using integrin-blocking antibodies we show that down-regulation of fibroblast proliferation by Thy-1 requires the interaction with $\beta 3$ integrins.

Moreover, transfection of the human Thy-1-negative fibrosarcoma cells HT1080 with Thy-1 significantly down-regulated proliferation and seeding HT1080 cells on recombinant Thy-1 induced a significant decrease of the proliferation rate. Taken together, these results emphasize the anti-proliferative effect of Thy-1.

In summary, beside its function as a cell-cell-adhesion molecule on activated endothelial cells, Thy-1 controls the proliferation and differentiation of fibroblasts, which is critical during wound healing and in the development of fibroblast-derived tumours. Thus Thy-1 seems to be a crucial surface marker controlling the balance between fibroblast proliferation and differentiation.

P034

Growth factors and cytokines are regulated by non-thermal atmospheric pressure plasma

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Non-thermal atmospheric pressure plasma is a forward-looking therapy option in medicine, especially in dermatology. It has been shown that eukaryotic cells could be stimulated and wound healing can be positively influenced by the application of plasma (Isbary et al. 2012; Metelmann et al. 2012).

In this study the effects of plasma were investigated on the human keratinocyte cell line HaCaT and the human monocyte cell line THP-1 as examples of wound healing relevant cells. Special focus was placed on the expression and secretion of wound healing related growth factors as well as inflammatory cytokines.

Cells were treated with a non-thermal atmospheric pressure plasma jet kinpen09 (neoplas GmbH, Greifswald). Subsequently the RNA was isolated and used for quantitative PCR (qPCR). Moreover secretion of growth factors and cytokines was detected by ELISA. Although the kinpen09 is typically operated with argon as a carrier gas, oxygen and nitrogen can be added at varying ratio. Hence reactive species in the plasma change, namely from ROS to RONS (Reuter et al 2012).

In order to determine a non-toxic plasma treatment time a cytotoxicity assay was performed employing CellToxTM Green Dye. Initially both cell types were treated with plasma for 20s and 180s. While the THP-1 cells reacted very robust towards the plasma treatment, HaCaT cells showed a dose dependent response. Accordingly treatment times were adjusted and expression as well as secretion of growth factors and cytokines was determined.

In this study it is presented that HaCaT keratinocytes express and secrete the growth factors VEGF and GM-CSF in a plasma treatment time- dependent manner. The amount of VEGF and GM-CSF can be controlled in accordance with the plasma composition. Similarly the expression and secretion of IL-8 and IL-6 depends on the plasma treatment time and the composition of the plasma as well. Contrarily, THP-1 monocytes express and secrete predominantly IL-8 in correlation with the duration of plasma exposure.

To our knowledge this study shows for the first time the expression and secretion of wound healing relevant growth factors and cytokine in HaCaT keratinocytes as well as in THP-1 monocytes. In addition the modulation of plasma components (varying oxygen and nitrogen concentration) can regulate their concentration. The secretion of wound healing promoting factors underlines the huge potential of plasma for wound healing and could give first insights in the underlying mechanisms on the cellular level.

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P035

The initiator caspase-10 protects from death receptor-induced cell death

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Death ligands are known to activate the extrinsic apoptotic pathway by processing initiator caspases like caspase-8 following formation of the death inducing signalling complex (DISC). Cleaved (active) caspase-8 is released and leads to subsequent apoptosis by effector caspase cleavage and activation. Caspase-10, a close homologue of caspase-8, was shown to be recruited to the DISC whereas its role in cell death signalling is controversial, and caspase-10 signalling is largely ignored.

To get further insights into the role of caspase-10 in cell death signalling, we performed knock down studies of caspase-10 by studying death ligand (CD95L or TRAIL)-mediated cell death in HeLa and HaCaT cells. Strikingly HeLa cells were sensitized to death ligand-mediated cell death, whereas HaCaT cells were not affected in their cell death response. Cell death was mainly apoptotic, because the pan-caspase inhibitor (zVAD-fmk) fully protected the cells whereas Necrostatin-1, an inhibitor of necroptosis, showed no protection. In contrast to HeLa, HaCaT cells express relatively high protein levels of caspase-10. The remaining caspase-10 protein levels after knock down in HaCaT cells are comparable to endogenous protein levels in HeLa, providing a potential explanation why HaCaT cells are not sensitized by the caspase-10 knock down. Caspase-10 protein levels in HeLa cells were not detectable by Western blot after the knock down. It is therefore a likely hypothesis that low levels of caspase-10 following knock down of this caspase in HaCaT cells are sufficient to protect from cell death.

To further prove this concept, a number of melanoma cell lines were screened for caspase-10 expression levels and demonstrated a markedly differential expression pattern of this caspase. Whenever caspase-10 was low, further downregulation of caspase-10 by siRNA sensitized to cell death, in line with our data in Hela cells. In contrast melanoma cells with high expression levels of caspase-10 were unaffected by knockdown of caspase-10. Taken together, our data surprisingly found a protective function of caspase-10 from death ligand-induced cell death. We will present further mechanistic insight how caspase-10 exerts its protective function and why low levels of caspase-10 exert sufficient protective activity to protect from cell death. We suggest that caspase-10 may serve as one predictive biomarker for death receptor agonist-induced tumor therapy.

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P036

Cutaneous hemorrhage during thrombocytopenia depends on neutrophil extravasation into the inflamed skin

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Platelets have been recognised as important mediators not only of hemostasis but also of vascular integrity during inflammation. It has been reported that in the absence of platelets inflammation leads to hemorrhage. In the present project we observed UVB-induced bleeding (Purpura solaris) in thrombocytopenic patients phototested for UVB tolerance (30–200 mJ/cm). In analogy, thrombocytopenic mice (anti-GPIbα) subjected to UVB-radiation developed cutaneous bleeding that occurred time- and dose-dependent and was strictly limited to the sites of irradiation. Moreover, UVB-radiation (100 mJ/cm) induced a significant influx of neutrophils as confirmed by analysis of the neutrophil-specific enzyme myeloperoxidase (MPO). To further understand the role of neutrophil recruitment in Purpura solaris, mice were treated with a neutrophil-depleting antibody (anti-Gr-1) prior to platelet depletion and UVB-radiation. Interestingly, under leukocytopenic conditions skin bleeding was virtually absent.

In two additional models of cutaneous inflammation, IC-mediated vasculitis (ICV) and irritative contact dermatitis (ICD), thrombocytopenic mice developed petechial bleeding as well. Despite profound tissue damage reduced neutrophil recruitment was observed during both ICV and ICD. By gradual interference with single steps of leukocyte extravasation, we finally identified neutrophils as the key inducer of thrombocytopenic bleeding during cutaneous inflammation in general.

Moreover, our findings indicate a dual role of platelets during cutaneous inflammation. On the one hand platelets support neutrophil recruitment and thus enhance inflammation, and on the other hand platelets secure vascular integrity and protect inflamed tissues from neutrophil-induced bleeding.

P037

Deletion of keratin K2 causes aberrant aggregation of K10, hyperkeratosis and skin inflammation

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Keratin K2 is one of the most abundant proteins of the epidermis, however, its biological significance has remained elusive. Here, we show that, in the mouse, K2 is expressed in the suprabasal epidermis of the ears as well as in segments of sole and tail skin but not in back skin. This expression pattern is exactly opposite to that of the other main suprabasal type II keratin, K1. Homozygous deletion of Krt2 caused hyperkeratosis, corneocyte fragility, elevation of trans-epidermal water loss and local inflammation in ear skin. Loss of K2 induced the expression of K1 in most cells of K2 knockout epidermis where it appeared to substitute for K2 in coexpression with the main suprabasal type I keratin, K10. K2 knockout cells, that failed to upregulate K1, developed massive aggregates of K10, nearly complete absence of a regular cytoskeleton and cytolysis. Conversely, K10 knockout mice showed granular clumping of K2 in ear skin. Taken together, this study demonstrates a non-redundant role of K2 at murine body sites with little or no protection by hair as well as a tendency of K2 and K10 to undergo clumping if expressed in an unbalanced manner.

P038

Production of reactive oxygen species and toxic effects of silver nanoparticles towards the human keratinocyte cell line HaCaT

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In medicine and health care silver is used as an antimicrobial and anti-inflammatory agent for example in silver containing wound dressings. Silver nanoparticles (AgNP) have also been studied over the last years to improve antibacterial effects by providing a slow but continuous release of silver. Although the antibacterial potential of AgNP has already been demonstrated, negative effects on mammalian cells and especially on skin cells have not been completely explored. It was reported that the toxicity of AgNP is due to the release of Silver ions (Ag+), but toxic effects due to nanoparticles themselves cannot be excluded. Ag+ induce an overproduction of reactive oxygen species (ROS) and therefore oxidative stress. The present study aims to investigate the mechanism of AgNP toxic effects on a human immortalized keratinocyte cell line (HaCaT) by means of techniques to detect ROS in cells and measurements of the cell viability (XTT assay). The release of Ag+ from AgNP is due to an oxidative reaction on the particle surface and takes place in the presence of oxygen. For a better understanding of the influence of Ag+ on ROS yield and cell toxicity, AgNP produced and stored under air (O2, with a high Ag+ content) were compared to those produced under argon (Ar, with a very low Ag+ content). As gold standard for measuring ROS the dichlorofluorescein (DCF) assay was used. Because an interference of AgNP with the fluorimetric DCF assay could not be excluded, additional experiments using electron paramagnetic resonance (EPR) spectroscopy were conducted. To our knowledge, EPR spectroscopy was used for the first time to show ROS production induced by AgNP on HaCaT cells. EPR spectroscopy outcomes were comparable to those of the DCF assay, but showed significantly smaller standard errors. Both techniques indicated increased ROS levels with a higher AgNP concentration with significant differences between the two AgNP types. The cell death correlated well with the detected levels of ROS. Moreover, it was found that the range of AgNP (O2) concentrations inducing only ROS was very close to those inducing cell death.

In conclusion, AgNP (O2) exhibited a higher toxicity than AgNP (Ar), suggesting that Ag+ significantly contributed to AgNP associated effects. Nevertheless, the toxicity found for AgNP (Ar) suggests that effects due to nanoparticles themselves might also exist. The results emphasize the complexity of the mechanisms underlining AgNP toxicity towards skin cells, which need to be carefully analyzed for a safe use of these nanomaterials in dermatology.

P039 (O03)

The role of Mcl-1 and Bcl-xL in homeostasis and function of murine mast cells

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Mcl-1 and Bcl-xL are anti-apoptotic Bcl-2 family proteins, which play essential roles during embryogenesis and are indispensable for survival of many cell types. Recent studies suggest that Mcl-1 and Bcl-xL also play a prominent role in the development and homeostasis of mast cells.

To explore the role of Mcl-1 and Bcl-xL in mast cells, we crossed Mcl-1 fl/fl and Bcl-xfl/fl mice (in which the Mcl-1 or Bcl-x genes can be deleted by a Cre recombinase) to the Mcpt5Cre strain, which expresses Cre selectively in connective tissue type mast cells. Mcpt5Cre/Mcl-1 fl/fl mice showed complete ablation of mast cells in various tissues, such as the dermis of back skin, mesentery, tongue, heart as well as submucosa and muscularis of the glandular stomach (<1% mast cells compared to control mice). We also observed significantly reduced mast cell counts in the peritoneal cavity and ears. In contrast, and as expected from the expression of the Cre transgene, the numbers of T cells, B cells, dendritic cells, macrophages and granulocytes were not altered, confirming that depletion of connective tissue type mast cells is highly specific. To investigate mast cell-specific responses in these mice, we performed experiments of IgE-mediated passive systemic anaphylaxis. As expected, Mcpt5Cre/Mcl-1 fl/fl mice were completely protected from the decline of body temperature, which is typically seen during anaphylaxis. In contrast to our observations in Mcpt5Cre/Mcl-1 fl/fl mice, Mcpt5Cre/Bcl-xfl/fl mice exhibited only a relatively minor reduction of mast cell counts in the tongue, heart and peritoneal cavity (40–55% reduction). Accordingly, there was no impairment of IgE-mediated passive systemic anaphylaxis in these animals.

Our studies define for the first time differential roles of Mcl-1 and Bcl-xL in the homeostasis of mast cells. The Mcpt5Cre/Mcl-1 fl/fl mice represent a new mast cell-deficient mouse model, which will be useful to specifically analyze functions of connective tissue type mast cells in immune responses and pathologic processes.

P040

What more do we want out of hair and from whom?

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At the age of non-invasive approach to regenerative as well as other treatments, harmless yield of starting cell material plays a crucial role in the development of a therapy. The small sample size, absence of pain, bleeding, wound or any kind of postoperative complications can make all the difference in acceptance of the progressing therapy by regulatory bodies, medical practitioners and patients.

Within such concept – particularly when it comes to autologous treatments – the importance of the mentioned benign sources grows and gains focus.

Perhaps paradoxical in the venture-gain sense, such source appears within a tiny hair follicle, or more precise its outer root sheath (ORS). This is, to the best of our knowledge, the smallest source of adult stem cells, among which are, at that, the naivest cells of the adult human body, able to give raise to many cell types. Those 'mother of all stem cells' as they are called, present the closest match to embryonic stem cells in terms of their developmental potency. Potential of this compact stem cell pool to differentiate into neurons, glia, melanocytes, keratinocytes, fibroblasts, chondrocytes, osteocytes, cardiomyocites, and endothelial cells has already been shown.

Mainly, our group at the Translational Centre for Regenerative Medizin / Dermatological Clinic At Leipzig University deals with the ectodermal and mesenchymal developmental potential of the ORS stem cell pool. We are currently focused on development of melanocytes and keratinocytes out of ORS for the purposes of non-invasive, autologous, transplantation-based therapies for wound healing and depigmentation, in particular Vitiligo. Standardized procedures have already been developed for quick, reliable and high yield cultivation of well defined, uniform primary cells available in antibiotic free sterile cultures based on explant culture of harmlessly plucked hair follicles. These procedures involve outgrowth on nylon meshes, favorable cultivation conditions for melanocytes, selection into pure culture and end characterization on protein and gene expression level.

Those cells have been tested within a number of biocompatible scaffolds and the procedures for the cultivation and application have been patented. In cooperation with Fraunhofer Institute for Cell Therapy and Immunology, enzymatic modifications of scaffold materials by lacase have brought about more adhesive and friendlier conditions for the melanocytes and keratinocytes and with use of cafeic acid and L-DOPA as substrates granted us very promising candidates for graft carrier.

Using similar procedures, we have cultivated cells that closely match mesenchymal stem cells out of human hair follicle ORS for the purposes of chondrogenic and osteogenic differentiation, aiming at regenerative therapies of cartilage and bone.

Further on, the procedures have been adjusted to physiologically fit cultivation conditions for explant cultures other than human – horse, chimpanzee and rabbit.

This abstract outlines not only our work in the field of ORS stem cells, but also that of others and comments on the entire vast regenerative potential of this elegant, nevertheless putative source.

P041

Alteration of epidermal calcium gradient is a key event in eczema formation

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(Atopic) eczema is characterized by altered skin barrier function, keratinocyte differentiation and proliferation. Calcium is thought to be a major player in regulation of epidermal differentiation and proliferation. To elucidate the fate and role of calcium in (atopic) eczema, we investigated the distribution of calcium in an atopic dermatitis like allergic dermatitis (AlD) mouse model in which we induced eczema of different severity. Calcium concentrations of ex-vivo samples were measured by 2-Photon Fluorescence Lifetime Microscopy (2P-FLIM). We correlated epidermal calcium distribution with different parameters of eczema such as epidermal thickness, level of infiltrate, IgE, and IgE-OVA, as well as markers of epidermal differentiation and epidermal barrier function. To elucidate putative regulators of calcium distribution we investigated its correlation with the expression/localization of tight junction proteins, connexins and calcium channels. We reveal an epidermal calcium distribution in native skin with a rise from stratum corneum (SC) to stratum granulosum (SG), a calcium peak within the SG, followed by a calcium decline to deeper epidermal layers and finally an area with low calcium concentration in the basal cell layer. In eczema, the overall amount of free calcium was only marginally changed, but the distribution was clearly altered, i.e. the calcium concentration in the SG was significantly higher and the area with low calcium concentration in the basal layers was increased. We found significant correlations between epidermal thickness, dermal infiltrate, IgE and transepidermal water loss with calcium parameters. Of note, there was a significant positive correlation between the area of low calcium concentration in the basal layers and expression of the marker of undifferentiated cells, K14, as well as proliferation, and a negative correlation with the early differentiation marker K10. Surprisingly, for the late differentiation marker Filaggrin downregulation in eczema was associated with higher calcium concentrations in the SG. Also tight junction (TJ) proteins were significantly correlated with Ca^{2+}-parameters and Ca^{2+}-channels and connexins were altered. In summary, we identify here alteration of calcium-distribution as a novel characteristic for eczema severity. Further, we show that there is a correlation of calcium and differentiation as well as proliferation also in 3D-tissues, but it is not found for all differentiation markers. Finally, we correlate paracellular, transcellular and intercellular parameters of Ca^{2+}-transport with the Ca^{2+}-profile of the skin.

P042

Positive effect of Myrtle oil solution on keratinocyte barrier function

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Myrtus communis is one of the traditional plants which were already identified by Persians, Greeks and other ancient civilized nations. It is traditionally used to treat fever and infections, to date, it is for example applied in acute and chronic respiratory infection, urinary tract infection, gum infections, herpes simplex (herpes type I and II) infections, acne and hemorrhoids. One of its proven effects is its antimicrobial / antifungal capacity. We wanted to know, whether there is an impact of Myrtus communis on cell viability of keratinocytes and whether its positive effects might also imply a strengthening of the barrier function of keratinocytes, thus helping to avoid the uptake of pathogens. Therefore we investigated on one hand a Myrtle oil solution (MOS; 10% in 72% (v/v) ethanol), which is commercially used in Iran for the treatment of herpes simplex, aphthae and impetigo contagiosa. The Myrtle oil was obtained by distillation of fresh leaves by steam or water. The essential oil which accumulates on the water is separated and subsequently diluted in ethanol to obtain the 10% solution. On the other hand, we used an ethanolic extract of dried Myrtus leaves (EAM) which was finally diluted in 96% ethanol (10 g extract/90 ml ethanol). Main components of both extracts are alpha-Pinen, Limonen and 1,8-Cineol, however, percentages and additional components differ. Both extracts were applied to primary keratinocytes undiluted and at 1:100, 1:500, 1:1000 and 1:5000 dilution in cell culture medium.

We investigated the short-term (20 h) and long-term (5 days) effect of the two extracts on viability of human primary keratinocytes. In short term experiments, undiluted MOS impaired cell viability in all and the 1:100 dilution in some cell populations while there was no or a slightly beneficial effect on cell viability with 1:500, 1:1000 and 1:5000 dilutions. EAM significantly reduced cell viability when undiluted and at 1:100 dilution in all cell populations and had no or a slightly beneficial effects at higher dilutions. In long term experiments, MOS impaired cell viability at 1:100 and 1:500, and did neither have a negative nor a positive impact at 1:1000 and 1:5000 dilutions. EAM impaired cell viability even at 1:5000 dilution. Subsequently, we investigated the effect of 1:1000 and 1:5000 dilutions of the MOS on keratinocyte barrier function (transepithelial resistance, TER) during calcium-induced barrier formation and after formation of the barrier. Applying MOS during barrier formation resulted in impaired barrier formation for the 1:1000 dilution while the 1:5000 dilution had no effect. When applied to an already formed barrier, the 1:5000 dilution had a positive effect on TER. In summary, our data show that myrtus communis has a positive effect on barrier function of keratinocytes, but the effect depends on the kind of extract, and the extract commonly used to treat e.g. herpes simplex has strongly to be diluted because of its negative effect on keratinocyte viability.

P043

Does mtDNA deletion accumulation lead to chronic inflammation during skin ageing?

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The process of chronic tissue inflammation, a hallmark of ageing, is multifactorial and the accumulation of DNA damage and protein aggregates, oxidative stress, as well as changes in the lipid composition of cellular membranes, are only some of the potential candidates causing an inflammatory response. Another hallmark of the ageing process is the accumulation of mitochondrial DNA (mtDNA) deletions in tissues. Organs of old individuals, like heart, muscle and brain display a mosaic of cells with normal or impaired mitochondrial function, depending on the load of deletions they harbour (Larsson, 2010). Given that increase in mtDNA deletion load and chronic inflammation in tissues are two phenomena which develop in parallel during ageing, a causal link between mitochondrial dysfunction and inflammatory response is an emerging concept, for which, however, little experimental data have been published so far (Green et al., 2011; Galuzzi et al., 2012).

In this context, the aim of our project is to determine whether there is relationship between accumulation of mtDNA deletions, and the development of a chronic inflammatory response, choosing skin as a model organ. For this purpose, we have established a mouse model which can express a severe dominant negative form of the mitochondrial replicative helicase TWINKLE (K320E-Twinkle) in keratinocytes via Keratin 14-cre recombination. This mitochondrial helicase is involved in mtDNA replication and has an active role in copy number control (Spelbrink et al., 2001). Patients harboring mutations in its gene accumulate deletions in various post-mitotic tissues over time.

Expressing this mutant helicase leads to the accumulation of mtDNA deletions and to mtDNA depletion in epidermis, as well as severe inflammation in the skin. Mutant mice were born in normal litter sizes according to Mendelian ratios. The animals showed growth retardation, hairless skin, a significantly lower level of blood glucose and significantly higher levels of lactate. Regarding the very short lifespan of the mutants (about 5 to a maximum of 8 days), we postulate lactic acidosis to be the cause of the early death. Analysis of the epidermal development with various differentiation markers revealed an overall normally differentiated epidermal compartment, however skin appeared thinner, contained less fat tissue and showed impaired development of skin appendages, like hair follicles. We observed a massive reduction in mtDNA copy number in epidermal sheets, resulting from the low efficiency of the K320E helicase, coupled with the high proliferation rate of keratinocytes. No hyperproliferative or apoptotic behavior of the mutant keratinocytes could be measured at birth and postnatal day 3. Keratinocytes of the epidermis showed mitochondrial dysfunction, as witnessed by cytochrome-c-oxidase negative cells, and the respiratory chain was only partially assembled. Despite this disturbed stoichiometry and the subsequent impaired enzymatic activity of the complexes, mitochondrial mass showed no impairment between controls and mutants.

Beginning from postnatal day 2 or day 3, all mutant animals start to develop scabs on the ventral skin and around the limbs and joints, accompanied by a severe inflammation, with macrophage and neutrophile infiltrates. Immunostainings of the ventral skin with different markers showed thickening of the epidermal sublayers, disorganized cellular layers and an impaired cell-to-cell contacts resulting in blister formation. Additional cytokine arrays experiments and skin barrier function tests should give more insights in the causality of these events and help in clarifying how mtDNA deletions themselves, and/or the resulting mitochondrial dysfunction, lead to the development of tissue inflammation.

P044

Do antimicrobially active C-terminal filaggrin-2 peptides kill bacteria via DNA-binding?

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Many cationic antimicrobial peptides kill bacteria by forming pores within bacterial membranes. A C-terminal cationic fragment of filaggrin-2 (FLG2-4), a structural protein of human skin, has been found to be highly active against preferentially soiland waterborne bacteria such as several *Pseudomonas* species as well as E. coli. To investigate the killing mechanism, we first performed transmission electron microscopy and found formation of blebs in FLG2-4-treated *P. aeruginosa*. To further analyze, whether bacterial lysis occurs via pore formation, the SYTOX Green assay was used. In this assay the dye, which is not cell-membrane permeable, increases its fluorescence when bound to accessible DNA as after cell disruption. Surprisingly, when *P. aeruginosa* ATCC 11446 was incubated with different concentrations of FLG2-4, the observed fluorescence declines with increasing concentrations of the peptide, suggesting a competition in DNA binding between the tested antimicrobial peptide and the DNA binding dye. Therefore we hypothesized that increasing FLG2-4 is blocking the dye binding and in consequence reducing fluorescence. Gel retardation assays with plasmid DNA confirmed the DNA binding capacity of FLG2-4, but clearly showed higher affinity for binding to linear or open circular, relaxed conformation than to supercoiled plasmid DNA. Relaxed DNA in bacteria is a required condition for replication, so it was assumed that FLG2-4 could interfere with bacterial DNA replication. As a short and simplified model for replication, FLG2-4 was tested on inhibiting properties in PCR reactions. Increasing FLG2-4 concentrations showed a decrease in band intensity for PCR products amplified from human skin cDNA. Taken together these results demonstrate that the antibacterial activity of the C-terminal fragment of FLG2 against *Pseudomonas aeruginosa* might affect bacterial growth through a mechanism that could involve DNA binding.

P045

Mammalian C-terminal filaggrin-2 peptides are soil-bacteria-killing antimicrobial peptides

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Human filaggrin-2 (FLG2) has been shown to be expressed in the outer layers of epidermis, mainly in the upper stratum granulosum and the stratum corneum. This protein shows the typical S100 fused-type protein structure with N-terminal S100- and EF hand domains followed by a spacer region, two types of repeats, and a C terminal region. We recently identified C-terminal peptide fragments as potent antimicrobial peptides (AMPs), preferentially killing Gram-negative bacteria like *P. aeruginosa* and other soil- and waterborne bacteria. An alignment of the hFLG2 C-terminus with those of other mammalian potential FLG2 orthologs reveals a stretch of about 24 highly conserved amino acids, which suggests an important and possibly similar function in these animals. To test the hypothesis that mammal FLG2 C-termini are antimicrobial peptides, two murine FLG2 C-terminal peptides spanning this conserved region were recombinantly generated as fusion proteins. An additional methionine was inserted between the tag and the protein sequence to cleave off the tag of the fusion protein chemically by cyanogen bromide. The purified, tag-free protein then was analyzed by radial diffusion assays, but did not show *E. coli*- and *P. aeruginosa*-killing activity. The lacking inhibition of bacterial growth implies that in mice the function of the FLG2-C-terminus seems to be different than in humans. To examine further if C-termini of other FLG2 putative orthologs are AMPs, peptides derived from sequences of other mammalian species covering the conserved C-terminal motif were synthesized and analyzed. These peptides include sequences obtained from mouse, cow, platypus, rabbit, and bushbaby. All of them exhibited *P. aeruginosa*-, *E. coli*- and the soil bacterium *P. stutzeri*-killing activity, with the latter being the most sensitive strain.

These findings indicate that FLG2 C-terminus peptides could be of importance for protection of the skin and mucosal surfaces from infection by ubiquitous soil- and waterborne bacteria. Thus, the presence of FLG2 could better explain, why environmental microbes usually are not able to infect us.

P046

N-terminal profilaggrin peptide fragments as contributors to the antimicrobial barrier of the skin

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Human skin and especially the stratum corneum as the outermost barrier of the body contain antimicrobially active compounds, which directly or indirectly control the growth of microorganisms and prevent infection. Besides hornerin and filaggrin-2 peptides we recently identified peptide fragments of the N-terminal region of profilaggrin (PFLG) in antimicrobially active HPLC-purified fractions of stratum corneum extracts. Due to the complexity of stratum corneum extracts and structural similarity of numerous N-terminal PFLG-peptides we had identified, it was not possible to purify single peptides to analyze and attribute antimicrobial activity to an individual molecule. HPLC-, ESI-MS-, and MS/MS-analyses of stratum corneum extracts revealed the presence of several PFLG peptides (FLG146–199, FLG146–200, FLG162–184, FLG162–207 and FLG176–207) in the antimicrobially active HPLC fractions. To test the hypothesis that these PFLG peptides, which originate from the PFLG N-terminal sequence also called the B-domain, may also represent antimicrobial peptides, the shortest peptide was synthesized, whereas the other four peptides were recombinantly expressed in E. coli as fusion proteins, containing either an full length SUMO tag or a shortened tag comprised only of a hexahistidine tag plus five further amino acids. In between the coding sequence for tag and protein an additional methionine codon was inserted to provide a cleavage site for a chemical cyanogen bromide scission. The expected tag-free peptides were then further purified by HPLC. The analyses for antimicrobial activity in a radial diffusion assay system are currently underway. Results will show, whether these defined peptides are AMPs – as some other N-terminal PFLG peptides – and thus may contribute, in addition to psoriasin and RNase-7, to the 'antimicrobial barrier' of healthy skin.

P047

Varying IL-8 responses in keratinocytes by clinical *Pseudomonas aeruginosa* isolates are associated with flagellin and rhamnolipid

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Pseudomonas aeruginosa is a ubiquitous gram negative bacterium of soil and aqueous habitats possessing a remarkable adaptability. From this point of view it is not astonishing that *P. aeruginosa* is frequently found on human skin and in human tissue infections.

While *P. aeruginosa* almost never infects healthy individuals, it can utilize breaks in the host defense systems to cause life-threatening infections. Here, impairment of the physical skin barrier, as in chronic wounds, surgery or burns, leads to prevalent entrances for this opportunistic pathogen. To reach preferred 'substrates', *P. aeruginosa* possesses a flagellum which provides motility and thus chemotaxis. The main structural protein of that whip-like appendage is flagellin. Interestingly, flagellin is a potent virulence factor that is recognized by the innate immunity of numerous organisms including men.

In previous studies we could demonstrate that *P. aeruginosa* releases flagellin from the flagellum with the help of its biosurfactant rhamnolipid. Presumably produced for accommodation to changed environmental conditions, these biosurfactants promote uptake and biodegradation of poorly water-soluble substances as well as motility on semi-solid surfaces. In turn, released flagellin is recognized by human keratinocytes, inducing the secretion of the proinflammatory cytokine IL-8. Since recognition by the host is usually detrimental for the pathogen, we were curious to investigate whether clinical isolates of *P. aeruginosa* modify their flagellin or rhamnolipid-mediated flagellin-release. To achieve this aim, *P. aeruginosa* isolates from different human sources were analyzed for the presence of flagellin, its IL-8-inducing potency in keratinocytes, and the ability to produce rhamnolipids. As results we found that all tested strains were motile, indicative for the presence of flagellin, and capable of producing rhamnolipids. Release of flagellin is accompanied with the onset of rhamnolipid-secretion in the stationary growth phase. In consequence the bacterial

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culture supernatants contain flagellin and induce the secretion of IL-8 in keratinocytes. Noteworthy, the detected rhamnolipid concentrations as well as the IL-8-inducing potency of the bacterial supernatants varied considerably among the tested isolates. Further investigation will identify possible impacts of rhamnolipids on keratinocytes to alter the IL-8 inducing potency of flagellin.

P048

A triterpene extract from the outer bark of the birch promotes wound healing under diabetic conditions

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Diabetes mellitus has become an increasing health problem over the last decades, and is considered one of the major concerns of the future. As a consequence of delayed wound healing, diabetic patients run an elevated risk for developing foot ulcerations. The latter may result in transtibial amputations, which cause both profound changes in patients' quality of life and financial challenges faced by the health care system. For these reasons, it is necessary to introduce treatment alternatives aiming at minimizing the risk of ulcer development as well as at promoting wound healing in diabetics. Triterpenes, natural compounds mainly produced by plants, are regarded to be suitable substances for these purposes. It was shown before, that a triterpene extract (TE) from the outer bark of the birch is beneficial for the treatment of superficial wounds in terms of healing progress and aesthetic outcome. Based on these results, it seemed promising to evaluate the effect of TE in a diabetic context, too. As test systems, we established, based on our patented *ex vivo* wound healing model for normal wounds, an *ex vivo* wound healing model to mimic diabetic conditions as well as control models for osmotic influences with mannitol. Models, which were cultured under diabetic conditions displayed a reduced capacity to heal when compared to both, the normal control and the osmotic controls. This reduced wound healing capacity is similar to *in vivo* diabetic wounds. Additionally, we developed an unwounded *ex vivo* model to test the potential of substances to alleviate changes caused by diabetic conditions and therefore prevent the formation of wounds. To this end, the same experimental setup was employed as above except for wounding the *ex vivo* models. We used two-photon microscopy to investigate the orientation of collagen and elastin fibres as markers for skin elasticity in biopsies cultured under diabetic in comparison with non-diabetic conditions. The angular distributions of collagen and elastin fibre orientation were altered in diabetic versus non-diabetic models, similar to what we observed in a subset of human diabetic tissue compared to human non-diabetic tissue. Interestingly, the osmotic controls often showed the changes found in diabetic models and human diabetic tissue. Of note, the skin of the wounded and non-wounded diabetic models, but not of the osmotic or the normal controls, was coloured yellow. This phenomenon can also be observed occasionally in the skin of diabetics. In the following, we analyzed the effect of TE using the wound healing model. The triterpene extract was applied to the wounds as ingredient of either an oleogel or a water-in-oil emulsion. For both formulations, the wound healing potential was significantly superior to PBS and comparable to that of the positive control PDGF under diabetic conditions.

P049 (O09)

Cytokine mediated induction of mTORC1 signaling prevents proper differentiation of keratinocytes and contributes to the pathogenesis of psoriasis

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Psoriasis is a chronic inflammatory skin disease that typically presents with sharply demarked, red scaly plaques that may be painful or itchy. Although biologics directed against cytokines, show promising results in the therapy of the disease, a comprehensive understanding of signaling mechanisms contributing to the pathogenesis is still missing.

Previously we could show that the PI3K/Akt pathway coordinates the equilibrium between proliferation and differentiation in keratinocytes and its deregulation contributes to psoriasis. Downstream of Akt the mTOR (mechanistic target of rapamycin) cascade is a major integrator of different signals and plays a key role in cell growth and homeostasis. As psoriasis also shows features of perturbed cell growth and differentiation, we investigated mTOR signalling in the disease and deciphered its contribution to the pathogenesis of psoriasis.

We found sign of high activity of mTOR signaling in the psoriatic epidermis. The mTOR kinase itself and components of the active mTOR1 complex (mTORC1) such as PRAS40 are hyper-activated in the psoriatic epidermis. We also found that the small GTPase Rheb, that is crucial for mTOR kinase activity is highly over-expressed in psoriasis. At the same time the downstream mediators of mTORC1, the S6 kinase, the ribosomal protein S6 and 4E-BP1 (eukaryotic initiation factor 4E (eIF-4E) binding protein-1) are hyper-activated in lesional psoriatic skin.

We then investigated *in vitro*, what factors mediate the activation of the mTOR pathway and found that psoriatic cytokines such as TNF-a, IL-1b and IL-17A are able to strongly induce S6 and 4E-BP activity. As a consequence of this activation, we found, that mTOR only partially mediates proliferative responses, but seems to play an important role in the regulation of epidermal differentiation.

We found that under healthy conditions, mTOR signaling is turned off as soon as keratinocytes start to differentiate. When we then simulated the psoriatic inflammation by treating keratinocytes chronically with IL-1b or TNF-a, mTOR signaling was continuously induced and proper differentiation was blocked. Similar results were obtained by hyper-activating the pathway through the over-expression of Akt

Conversely, regular differentiation could be restored under these inflammatory conditions, if mTOR signaling was blocked using rapamycin or siRNA mediated knockdown of mTORC1 components. This argues that in psoriasis the constant activity of mTORC1 is responsible for the differentiation defect that contributes to plaque formation.

In summary, our data suggest that cytokine induced activation of the Akt/mTOR cascade contributes to the induction and/or maintenance of the psoriatic phenotype through the induction of proliferation and blockade of proper differentiation, thus pointing towards mTOR as a potential target for therapeutic intervention in psoriasis. mTOR is efficiently inhibited by rapamycin (sirolimus), which is a pharmacologically well-established drug and first trials have already shown promising results in psoriasis. Thus our results suggest to further explore topical formulations of mTOR inhibitors for anti-psoriatic therapy.

P050 (O29)

Nox4 is an intrinsic mediator of transforming growth factor-beta1-induced fibroblast activation and a target for the neuropeptide alpha-melanocyte-stimulating hormone

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Transforming growth factor-beta1 (TGF-beta1) is a master regulator of collagen synthesis and fibrosis. Here, we show that Nox4, a member of the 7 nicotinamide adenine dinucleotide phosphate oxidase (Nox) homologues, is the exclusively expressed Nox isoform in human dermal fibroblasts (HDFs). Expression of the Nox4 adaptor proteins p22phox and Poldip2 which regulate Nox4 enzyme activity and stability was also detected in these cells. TGF-beta1 significantly upregulated Nox4 mRNA expression in a time- and dose-dependent manner and this effect was due to transcriptional induction. Nox4 induction by TGF-beta1 was confirmed by Western immunoblotting and immunocytochemistry. Mechanistically, this effect of TGF-beta1 on Nox4 expression in HDFs was dependent on functional SMAD signaling as shown by preincubation with the SMAD3 inhibitor SIS3. Using a combined approach, i. e. pharmacological inactivation of Nox4 with the pan-Nox inhibitor diphenyleneiodonium and gene suppression by Nox4 siRNA we could demonstrate that TGF-beta1-mediated synthesis of collagen type I (COL(I)) but also induction of the myofibroblast markers -smooth muscle actin (alpha-SMA) and fibronectin (FN 1) depend on functional presence of Nox4. In a final set of experiments we wondered if the neuropeptide -melanocyte stimulating hormone (alpha-MSH), which previously had been shown to antagonize TGF-beta1-mediated collagen synthesis and to reduce experimentally induced skin fibrosis, acts via Nox4 to exert its beneficial effects. Alpha-MSH in fact suppressed TGF-beta1-mediated expression of Nox4, and consequently induction of both COL(I), alpha-SMA and FN 1. Our results show that Nox4 is a novel intrinsic regulator of the activated state of human dermal fibroblasts induced by the profibrotic cytokine TGF-beta1. Moreover, downregulation of Nox4 appears to be a new effector mechanism of the neuropeptide alpha-MSH in the context of protection against oxidative and fibrotic stress responses.

P051

Superoxide anions induce IGF-1 resistance through concomitant activation of the key phosphatases PTP1B and PTEN in fibroblasts *in vitro* and in aged skin *in vivo*

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The regulation of the evolutionary conserved insulin/IGF-1 signaling pathway is critically involved in longevity, metabolism, tissue homeostasis as well as cancer suppression and depends on the delicate balance between activating kinases and suppressing phosphatases at different steps of insulin/IGF-1 downstream signaling. We here report the novel finding that accumulation of superoxide anion radicals (O_2) in the mitochondria as studied in rotenone treated dermal fibroblasts or in the connective tissue specific manganese superoxide dismutase (SOD2) deficient murine model for premature aging results in a significant activation of two key phosphatases, PTP1B and PTEN, eventually dampening the IGF-1 induced signaling cascade via dephosphorylation of specific tyrosine residues of the IGF-1 receptor (IGF-1Rβ chain) and phosphatidylinositol 3,4,5-trisphosphate (PIP3), respectively. Dephosphorylation of these key targets resulted in reduced activation of PI3 kinase, ribosomal S6 kinase and AKT as determined by Western blot analysis and immunostaining of skin sections derived from the connective tissue specific SOD2 deficient mice. The specific inhibition of PTP1B and PTEN either by shRNAs or by small molecule inhibitors abrogated the O_2 – induced IGF-1 resistance further underlining the specific involvement of PTP1B and PTEN. The IGF-1Rβ chain and downstream effectors in their dephosporylated inactive state are suspected to supress cell growth by dampening the biosynthesis of translational components and components of the extracellular matrix. In fact, we found that the O_2 – dependent IGF-1 resistance resulted in decreased proliferation of murine dermal fibroblasts and significantly reduced mRNA levels of α1 (I), α1 (III), and α2 (I) collagen chains, molecular hallmarks of skin aging. These data are clinically relevant as in replicative senescent human fibroblasts, superoxide anion radical concentrations are critically increased and skin sections from old human individuals showed more oxidative damage compared with young individuals. Notably, the IGF-1 signalling pathway was found to be down-regulated in the skin of elderly individuals. Collectively, these data suggest that O_2, PTP1B and PTEN represent promising targets for drug development to prevent and treat skin aging and age-related disorders driven by persistent insulin/IGF-1 resistance.

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P052

Epidermal cFLIP regulates skin homeostasis and protects adult skin from TNF-induced keratinocyte apoptosis: beneficial effect of TNF-R2-Fc

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FADD, caspase-8, and cellular FLICE-inhibitory protein cFLIP play a key role in regulation of the outcome of cell death signalling. Mice that constitutively lack any of these molecules die at early embryonic age. However tissue-specific deletion of either FADD or caspase-8 resulted in inflammatory skin disease caused by increased necroptosis. cFLIP function for the control of inflammatory and cell death pathways *in vivo* is unknown.

We have generated mice lacking cFLIP in the epidermis. In contrast to caspase-8 knockout, we show that mice with constitutive epidermal-specific deletion of cFLIP die around E10.5. To analyse the role of cFLIP in adult animals, we next generated mice with skin-specific inducible postnatal deletion of cFLIP.

Shortly after tamoxifen application, cFLIP expression was abrogated in adult skin of cFLIPfl/fl-K14CreERtam mice. Loss of cFLIP resulted in severe macroscopically detectable inflammation of the skin in tamoxifen-treated animals, but not in control animals. Histological, immunohistological and *in vitro* analysis revealed rapid caspase activation and apoptotic, but not necroptotic cell death in the skin or cultured keratinocytes. Our *in vitro* system demonstrated that TNF mRNA induction and protein secretion occur as a response to the loss of cFLIP arguing that a TNF-mediated autocrine loop in the skin is the cause of TNF-mediated apoptosis of cFLIP-deficient PK *in vivo*. Furtermore *in vitro* and *in vivo* inhibition experiments using TNF-R2-Fc fusion protein revealed the critical functional role of TNF, for epidermal cell death.

Of note epidermal cFLIP protein was lost in patients with severe drug reactions associated with epidermal apoptosis.

Taken together our findings provide new insights into the regulation of cell death machinery in human and murine epidermis and warrant future studies to dissect the impact of cFLIP loss in a number of inflammatory and neoplastic diseases of the skin.

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P053

The adipokine vaspin – anti-inflammatory action on leukocytes

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Here, we propose a role for the serpinA12, vaspin, expressed in the epidermal layer of skin for the control of inflammatory processes.

Originally, vaspin expression was found in adipose tissue of obese individuals. Recently, we identified keratinocytes in the skin as a major source of vaspin. Here, we show for the first time that the expression of vaspin in keratinocytes correlates with the differentiation of keratinocytes. In contrast, induction of proliferation and stimulation with pro-inflammatory mediators attenuate vaspin expression. Accordingly, in psoriatic skin lesions which are associated with disturbed differentiation and proliferation of keratinocytes and infiltration of inflammatory cells, expression of vaspin is clearly diminished. To analyze the effect of vaspin expression in keratinocytes on the control of inflammatory reactions the keratinocyte-like cell line HaCaT was transfected with vaspin resulting in a model system with keratinocyte-like cells with high expression of vaspin (HaCaT-vaspin) and control cells which lack vaspin expression (HaCaT-ctr vec). In the present study we demonstrated a repression of activation of different subtypes of leukocytes resulting in a decreased secretion of proinflammatory cytokines like TNFα and IL-6 as well as of chemokines such as MCP-1 and IL-8 from leukocytes by vaspin expressed in the keratinocyte-like cell line HaCaT. The physiological importance of the vaspin-mediated immune suppression was demonstrated in a mouse model of a psoriasis-like skin inflammation. In the presence of vaspin the infiltration of neutrophils and macrophages was clearly down-regulated indicating the anti-inflammatory action of vaspin. The analysis of the underlying mechanisms revealed that vaspin might act directly via a still unknown receptor. More interestingly, due to the fact that recombinant vaspin showed a clear less effect than vaspin expressed by HaCaT-cells we suggested an indirect action of vaspin. Indeed, the analysis of vaspin expressing HaCaT-cells revealed strong differences to the control cells including an increase in cell-cell-contacts, decreased migration and higher expression of E-cadherin and HAS2. Genetic deletion experiments showed that the inhibition of TNFα secretion by DC upon coculture with HaCaT-vaspin is dependent on the expression of HAS2 and E-cadherin on HaCaT-vaspin.

Taken together, vaspin expressed in keratinocytes displays an anti-inflammatory action on different subsets of leukocytes via two separate ways. First, vaspin might bind to an until unknown receptor to mediate its effects. On the other hand, vaspin seems to be involved in the control of several functions of keratinocytes which in turn regulate the functional capacity of leukocytes. Both, direct and indirect action of vaspin results in down-regulation of inflammatory reactions.

P054

Classical cadherins control epidermal barrier function: regulation through actomyosin?

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Intercellular junctions are crucial determinants of tissue architecture and integrity. Previously, we have shown that epidermal deletion of E-cadherin results in trans-epidermal water loss and subsequent perinatal death of mice due to improper functioning of epidermal tight junctions (TJ). How E-cadherin regulates epidermal TJ is not known. We first asked whether this is a specific function of E-cadherin. Re-expression of either E-cadherin or P-cadherin, the only other classical cadherin expressed in the epidermis rescued tight junctional barrier formation in primary E-cadherin−/− keratinocytes, indicating that overall level but not specificity of classical cadherins is important. We next asked whether classical cadherins regulate the recruitment of the tight junctional components to intercellular contacts. Surprisingly, membrane recruitment of key TJ proteins, such as ZO-1 and claudins to tight junction-like structures was not obviously disturbed in E-cadherin−/− cells. More importantly, ultrastructural freeze fracture analysis revealed the formation of TJ strands, the presence of which was considered an indication of barrier function. Thus, our system allows us to separate initial TJ assembly from barrier function, and the data suggest that classical cadherins regulate a late step in the biogenesis of TJs. We asked whether classical cadherins control the mechanical force exerted by the actomyosin cytoskeleton on tight junctions. Cadherins were recently shown to mediate mechanotransduction in an alpha-catenin- and vinculin-dependent manner. Vinculin staining revealed a strong reduction in the stratum granulosum of E-cadherin−/− epidermis, where the tight junctions reside, whereas in cultured keratinocytes the distribution of ZO-1 and vinculin was altered upon loss of E-cadherin. This was accompanied by changes in Rac activity and an altered distribution of the actomyosin cytoskeleton. Traction force microscopy analysis revealed decreased intercellular adhesion force upon loss of E-cadherin indicating that transcellular coupling of contractility is affected. At the moment we are performing rescue experiments with different cadherin mutants to determine the structural requirement for classical cadherins in epidermal barrier function. Overall our data show a crucial role for E-cadherin in epidermal barrier function, most likely by orchestrating actomyosin contractility within the epidermal sheet.

P055

Adhesion maturation of neutrophils on nanoscopically presented integrin ligands

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Aim: Integrins and their respective ligands are critical for many cellular functions in health and disease, including leukocyte adhesion, migration and cell signaling. The integrin Mac-1 (CD11b/CD18), for example, plays a fundamental role in cardiovascular disease as it enables neutrophilic granulocytes to interact with the platelet receptor GPIbα on platelet aggregates. In spite of the importance of these receptor-ligand interactions, *in vitro* systems that allow the precise control over biophysical parameters like ligand density and orientation as well as shear stress have not been available so far. Therefore, our aim was to develop a precisely tunable biomimetic system to study receptor-ligand interactions. In this setup, we then quantitatively determined the conditions for Mac-1-dependent neutrophil adhesion on human GPIbα.

Method: The distribution of GPIbα on a platelet surface was mimicked by using a combined nanopatterning/biofunctionalization approach. Nanopatterns of 6 nm gold nanoparticles were assembled on glass surfaces by block copolymer micellar nanolithography (BCML) and subsequently served as anchor points for site-directed immobilization of the integrin ligand GPIbα. With this method, the distance between individual biomolecules can generally be adjusted in the range between 25 nm and 300 nm. Here, GPIbα interparticle spacings of 60 nm, 100 nm and 200 nm were used, reflecting the biological range of GPIbα densities under different physiological and pathophysiological conditions. For the control of hydrodynamic parameters, the glass substrates were integrated into a microfluidic system. Neutrophil behavior on GPIbα-nanostructured surfaces was then surveyed and quantitated.

Results: We have developed a novel approach to study receptor-ligand interactions in an *in vitro* system that, unlike most conventional nanopatterning methods, provides control over hydrodynamic conditions as well as the local receptor density. When interacting with nanoscopically presented GPIbα under physiological flow conditions, neutrophils require a minimum spacing of GPIbα molecules to successfully adhere. In contrast, under low-flow conditions, neutrophils adhered on all tested GPIbalpha densities with subtle but non-linear differences in cell response, including spreading behavior, adhesion maturation and mobility. Surprisingly, neutrophil adhesion on GPIbα was very robust to density variations up to 1 order of magnitude. Our results reveal how neutrophils differentially process the number of available surface receptors on the nanoscale and show how adhesion and adhesion maturation are regulated by GPIbαdensity. In the future, this model system can be useful to determine optimum therapeutic ranges for targeting integrin-dependent interactions.

P056

Desmosomes assembly: a classical cadherins-dependent process

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Adherens junctions and desmosomes are calcium-dependent adhesive complexes necessary to establish and maintain intercellular interactions. Using mouse keratinocytes deficient for the adherens junction components E- and P-cadherin, we have previously shown that classical cadherins are key regulators of desmosomes assembly. The underlying mechanisms why desmosomal cadherins require classical cadherins to establish stable cell-cell contacts are not known. We thus asked how classical cadherins control desmosomes formation. Cells lacking classical cadherins presented reduced total and cell surface levels of different desmosomal proteins but did not alter their internalization rate. Pharmacological inhibition of Epidermal Growth Factor Receptor restored desmosome assembly and increased total and cell surface desmosomal cadherins suggesting that classical cadherins control desmosome formation by regulating desmosomeal cadherin levels. However, overexpression of desmosomal cadherins did not rescue desmosomes. Through structure/function analysis we next investigated the molecular requirements for classical cadherins necessary for desmosome assembly. Using a combination of cadherin deletion and chimeric mutants, we could show that desmosome assembly by classical cadherins required a combination of the cadherin extracellular domain, the juxtamembrane domain and the linkage to α-catenin. Interestingly, the extracellular domain of Dsg2 when fused to the E-cadherin cytoplasmic tail and vice versa, the E-cadherin extracellular domain fused to either Dsg2 or Dgs3 cytoplasmic region could not promote desmosomes nucleation, implying unique properties of classical cadherin in regulating this process. Overall our results suggest that classical cadherins need to engage in adhesion and interact with the catenins for desmosome assembly, perhaps by coordinating the transport to the cell surface of desmosomal complexes.

Chemokines/Cytokines

P057

The aryl hydrocarbon receptor (AHR) cooperates with NF-κB to induce IL-6 expression in UVB-exposed keratinocytes

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Acute exposure to ultraviolet (UV) radiation induces a variety of inflammatory skin reactions, such as erythema, neutrophil infiltration, vascular remodeling, and secretion of inflammatory mediators. Keratinocytes (KC) are the major epidermal cell-type responsible for the release of pro-inflammatory cytokines, such as IL-8, TNFα, and IL-6. The expression of IL-6 is up-regulated in several inflammatory skin diseases (atopic dermatitis, psoriasis) and is also involved in the pathogenesis of skin cancer.

Here we identify the AHR, a key regulator of xenobiotic metabolism, as a novel modulator of UVB-induced IL-6 expression. In contrast to AHR-proficient NCTC 2544 KC, quantitative gene expression analyses revealed a reduced increase (~50%) of IL-6 in AHR-knockdown KC 6 h after exposure to 200J/m2 or 400J/m2 UVB. Accordingly, ELISA-based measurements showed a significant decrease (~40%) of secreted IL-6 in the supernatant of AHR-knockdown KC 24 h after exposure to 400J/m2 UVB.

The AHR is activated by tryptophan photoproducts generated in the cytosol of UVB-exposed KC. Upon activation, the AHR shuttles into the nucleus, dimerizes with ARNT, and binds to so-called xenobiotic-responsive elements in the promoter of target genes (e.g. cytochrome P450 1A1) to enforce transcription. The AHR can also interact with other transcription factors, for instance the NF-κB subunit RelA. Interestingly, NF-κB is the major regulator of IL-6 expression in KC. To test if the AHR interacts with NF-κB signaling to induce IL-6, we pre-treated AHR-proficient and -knockdown KC with BAY 11-7085, a potent NF-κB inhibitor, prior UVB exposure. The UVB-induced IL-6 induction was completely abrogated by BAY 11-7085 in both cell-types, demonstrating the dominant role of NF-κB in IL-6 regulation. A combined exposure of AHR-proficient cells to UVB radiation and lipopolysaccharide caused an additive IL-6 induction, which was significantly less developed in AHR-knockdown keratinocytes. Importantly, electrophoretic mobility shift assays (EMSA) performed with nuclear extracts of UVB-exposed KC revealed a concomitant binding of AHR and RelA to the NF-κB binding site located in the human IL-6 promoter. Thus, it is highly likely that the AHR enhances IL-6 expression by cooperating with NF-κB, thereby probably contributing to the development of UVB-induced inflammatory reactions and associated skin diseases.

P058

Epidermal CCL27 expression is regulated during skin development and keratinocyte differentiation

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Adult human skin contains a large number of memory T cells which are attracted by the constitutively expressed chemokine CCL27. In contrast, prenatal skin harbors only few memory T cells. In this study we examined whether this scarcity correlates with CCL27 levels during gestation *in vivo* and analyzed its expression in fetal and adult human primary keratinocyte (KC) as well as organotypic skin cultures *in vitro*.

Immunofluorescence revealed no/low CCL27 expression in embryonic (9–14 weeks estimated gestational age (EGA)) and fetal (18–24 weeks EGA) human skin, respectively, as compared to the strong staining pattern observed in adult skin. Consistent with this *in situ* expression pattern, secreted CCL27 was present in supernatants of ex-vivo skin cultures derived from adult skin samples but was absent in supernatants of prenatal skin. Similarly, CCL27 was produced and secreted *in vitro* by adult primary human KC but not in fetal primary human KC. Stimulation with the TLR3 ligand poly (I:C) – a potent inducer of a variety of chemokines in KC – led to a strong induction of CCL27 secretion merely in adult but not in fetal KC. Given that a major difference between pre- and post-natal epidermis is the differentiation status of KC, we investigated the effect of KC-differentiation on CCL27 production and secretion in monolayer and organotypic skin cultures using adult KC. In both experimental settings, cell-differentiation strongly up-regulated CCL27 expression and secretion in KC.

All together our findings suggest that CCL27 plays a major role for the influx of memory T cells during skin development. In addition, we demonstrated that epidermal CCL27 secretion is strongly dependent on KC differentiation.

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P059

Human dermis-derived ABCB5-positive mesenchymal stem cells improve wound healing in a mouse model for chronic venous ulcers

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Mesenchymal stem cells (MSCs) feature many characteristics, such as tissue regeneration capacity and immune modulation, beneficial for therapeutic applications in injury and trauma. With respect to skin wound healing, MSCs have been proposed to suppress inflammatory processes and stimulate repair mechanisms such as myofibroblast differentiation and matrix deposition, angiogenesis, as well as re-epithelialisation. Although MSCs are present in all connective tissues of the body including the dermis, these studies have mainly focused on MSCs isolated from either bone-marrow or adipose tissue. Here we describe the isolation of an ATP-binding cassette sub-family B member 5 (ABCB5) positive plastic-adherent dermal cell subpopulation and its characterization as bona-fide MSCs. ABCB5+ dermal MSCs contributed to full-thickness excisional skin wound healing in mice to a comparable level as GMP-isolated and cultured bone-marrow derived MSCs. Furthermore, we demonstrated that ABCB5+ dermal MSCs secreted interleukin-1 receptor antagonist (IL-1RA) in response to inflammatory stimulation, which in turn inhibited classical (M1) macrophage activation with TNF-α release. IL1-RA inhibits the activity of IL-1 cytokines by binding to the IL-1 receptors without activating signal transduction. The importance of MSC-secreted IL-1RA for the observed accelerated cutaneous wound healing in mice was substantiated by a siRNA-mediated gene-silencing approach. As excessive and persistent M1 macrophage activation is a hallmark of many non-healing wounds, we further explored the effect of anti-inflammatory dermal ABCB5+ MSCs treatment in an iron-overload mouse model for chronic venous ulcers. The results indicate a beneficial effect of this cellular therapeutic approach on wound closure as well as scar quality. In conclusion, human dermal ABCB5+ sorted MSCs represent an easy accessible source for cell-based therapy of skin wounds that ameliorates healing at least in part by the secretion of the anti-inflammatory factor IL-1RA.

P060

Are RNA aptamers suitable therapeutic tools for topical treatment of inflammatory skin diseases?

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RNA aptamers are valuable therapeutic tools that have advantages over systemic treatments with antibody based biologics. RNA aptamers show high specificity and affinity, low immunogenicity, and can be less costly compared to antibody therapies.

The aim of this study was to investigate the potential of a previously described aptamer with neutralisation capacity against IL-17A on pro-inflammatory mediator release by skin resident cells. This was carried out with recombinant protein and also in a co-culture with IL-17 producing T cells (CD4+CCR6+).

The aptamer showed a good neutralising effect on human primary dermal fibroblasts. Surprisingly, that effect was not seen in keratinocytes stimulated with recombinant IL-17 or co-cultured with activated CD4+CCR6+ T cells. We were able to show that this lack of activity in keratinocyte cultures was due to rapid internalisation of the aptamer. This was confirmed by immunofluorescence with different labelled aptamers.

This finding is of interest as the efficient aptamer uptake capacity of primary human keratinocytes could be used as a tool for intracellular targets without the need for chemical permeabilisation of the cell.

P061

CCL7 (macrophage chemotactic protein-3, MCP-3) is upregulated in lesional psoriatic skin in a TNF-alpha dependent fashion

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Chemokines are small chemotactic proteins that have a crucial role in leukocyte recruitment into tissue. Targeting these mediators has been suggested as a potential therapeutic option in inflammatory skin diseases.

Using quantitative PCR, we searched for transcripts of proinflammatory mediators in psoriatic skin. We found that CCL7 (monocyte chemotactic protein-3, MCP-3), a chemokine ligand known to interact with multiple C-C chemokine receptors, was markedly increased in lesional as compared to nonlesional and normal control skin (40.2 and 63.6 fold increase, respectively; $P < 0.001$). Surprisingly, its expression outnumbered mRNA levels of other C-C chemokines known to be upregulated in lesional psoriatic skin, namely CCL2 (MCP-1), CCL3 (MIP-1A), CCL5 (RANTES), CCL11 (Eotaxin-1), CCL19 (MIP-3B), CCL20 (MIP-3A) and CCL22. In contrast, we did not detect increased levels of CCL7 in other inflammatory skin diseases such as atopic dermatitis or lichen planus.

Immunohistochemistry revealed a strong CCL7 staining signal in blood endothelial cells of lesional psoriasis skin as opposed to healthy control skin and atopic dermatitis.

In cultured human blood endothelial cells, CCL7 protein expression could be induced by TNF-alpha, but not by IL-4, IL-10, IL-13, or IL-6. Conversely, CCL7 mRNA expression in lesional skin of psoriasis patients was decreased to levels found in nonlesional skin already 16 h after a single intravenous infusion of the TNF-alpha-blocker infliximab.

In sum, our data point to a pathophysiological role of CCL7 (MCP-3) in the development and maintenance of psoriatic skin disease.

P062

Fibroblasts contribute to the increased expression of CXCL5 in human skin after UV exposure

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The ability to detect and respond to noxious stimuli is a crucial function of the peripheral nervous system in the skin. Overexposure to UV radiation induces local inflammation, skin damage and primary hyperalgesia. The mechanisms contributing to mechanical or heat hyperalgesia are poorly understood. Recently, the chemokine CXCL5 was identified to be upregulated in skin biopsies after UVB treatment. Furthermore, CXCL5 was shown to induce mechanical hyperalgesia in rats. However, little is known about regulation of CXCL5 in human keratinocytes and fibroblasts after UV treatment. We presumed that, in addition to immune cells, skin cells also contribute to elevated CXCL5 levels. The UV-induced regulation of CXCL5 was studied in human 3-dimensional organotypic skin cultures, human primary keratinocytes and fibroblasts. Our results show an increased CXCL5 secretion in organotypic skin cultures, comprising keratinocytes and fibroblasts, after irradiation with solar simulated light (SSL). Interestingly, when analyzing UV-induced CXCL5 expression in detail, only fibroblasts and not keratinocytes showed an increased expression of CXCL5 mRNA and protein. Furthermore, our data reveal that the CXCL5 receptor CXCR2 is expressed in keratinocytes, fibroblasts and DRG neurons. Therefore, we propose that elevated CXCL5 levels in human skin might have a direct influence on DRG neurons, keratinocytes and fibroblasts.

P063

The S100 'alarmin' psoriasin controls epidermal inflammasome activity and IL-1β release in psoriasis

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IL-1β is a potent player in cutaneous inflammation and important for the development of the Th17 micro-milieu in autoinflammatory diseases. Its activity is controlled on transcriptional level and by subsequent proteolytic cleavage by inflammasome complexes. Recently, the NALP1 inflammasome has been genetically linked to Th17-mediated autoinflammatory diseases including psoriasis. However, the regulators and mechanisms which facilitate NALP1 inflammasome-induced IL-1β production in non-infectious diseases are unknown. S100 peptides have been identified as danger-associated molecular patterns abundant in the psoriatic epidermis. Here we report the NALP1-inflammasome is active in human epidermal keratinocytes and increased in psoriatic skin lesions. Like TNFα, the S100 peptide psoriasin facilitated the DNA-mediated IL-1β production by keratinocytes compared to koebnerisin (S100A15), which is highly homologous to psoriasin but functionally distinct. The effect of psoriasin is mediated via up-regulation of caspase-1 and caspase-5 as it does not activate inflammasomes by itself. IL-17A further amplified the expression of psoriasin and its regulatory effect on the NALP1 inflammasome. It additionally affected the expression of NALP3 and AIM2 and suppressed the IL-1β release by epidermal keratinocytes. Thus, our data identify psoriasin as danger-associated molecular patterns for NALP1 inflammasome activity in psoriasis, proposing new molecular targets in Th17-mediated autoinflammation and autoimmune skin diseases.

P064

The chemokine CXCL14 acts as a modulator of CMV dissemination

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Human cytomegalovirus (HCMV) is an ubiquitous betaherpesvirus infecting between 70 and 100% of the adult population worldwide. It is a leading cause of virus-associated birth defects and it causes severe and fatal diseases in immune-compromised individuals, e.g. transplant recipients and HIV patients. HCMV enters the host at epithelial surfaces, but for its persistence and latency it requires systemic dissemination. Although these mechanisms remain largely elusive, monocytes are suspected to be the main carriers of viral dissemination.

Chemokines are redundant secreted proteins with growth, differentiation, and activation functions that regulate and determine the nature of immune responses and control immune cell trafficking. One homeostatically expressed chemokine in the skin is CXCL14, known for its chemotatic effect on monocytes. Thus, we investigated the effect of HCMV infection of human fibroblasts (MRC-5) and indeed could show that the expression of the chemokine CXCL14 is dramatically upregulated after infection. Therefore, we propose that HCMV hijacks the chemokine-driven host response to infect monocytes as well as immature dendritic cells which in turn amplify and spread HCMV progeny.

To further investigate this hypothesis, we infected C57BL/6 mice intradermally with MCMV to analyze the way of infection via real-time PCR and plaque-essays *in vivo*. Thereby, we discovered an altered pattern of HCMV dissemination. We demonstrated that in the case of an intradermal injection of MCMV the virus mainly spreads into the salivary glands. Taken together, these results suggest a novel chemokine driven mechanism of HCMV pathogenesis and a more authentic dissemination process *in vivo*.

P065 (O13)

Treatment of IL-23-dependent autoimmune disease by RNAi targeting dendritic cells

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Previous reports suggest that dendritic cells (DC) are resistant for spontaneous uptake of small interfering RNA (RNAi) under *in vitro* conditions. However, certain modifications allow DC to incorporate biologically efficient amounts of RNAi. We constructed a series of synthetically-modified RNAi (smRNAi) sequences for specific silencing of the interleukin (IL-)12/IL-23 subunit p40 to treat autoimmune diseases. Two of our p40-smRNAi constructs reproducibly silenced LPS-triggered IL-12p70 production by DC. To test the spontaneous incorporation of the smRNAi we generated carboxyfluorescein (CF)-labeled and 33P-labeled constructs. First, we studied the uptake of CF-labeled p40-smRNAi by DC *in vitro* using fluorescence microscopy and flow cytometry. Intracellular fluorescence was visible and could be quantified when DC were incubated with CF-labeled p40-smRNAi. In contrast, fluorescence was mostly absent when DC were incubated with CF-labeled non-modified p40-RNAi. *In vivo*, we followed the 33P uptake by different organs and cell types after injection of 33P-labeled RNAi. Interestingly, we could detect enrichment of 33P signals in lymphoid tissues. When administering 33P-labeled p40-smRNAi to mice, a significant enrichment was detected in CD11c-positive cells, compared to mice receiving the non-modified 33P-labeled p40-RNAi. The uptake of the p40-smRNAi by DC was confirmed by functional assays. Only p40-smRNAi was capable to suppress p40 expression in activated DC, while non-modified p40-RNAi or control-smRNAi showed no significant changes in p40 expression. The expression of unrelated cytokines remained unaffected. Further, *in vivo* our p40-smRNAi construct inhibited the generation of interferon γ or IL-17 producing Th1 and Th17 cells after specific immunization. Administration of p40-smRNAi to mice immunized for experimental autoimmune encephalomyelitis was capable to protect the mice from severe disease. In mice treated with p40-smRNAi we could observe a suppression of Th1 and Th17 responses in peripheral as well as CNS-infiltrating T cells. Thus, our p40-smRNAi could be used as novel targeted immunotherapy for autoimmune diseases.

P066

Induction of TNFAIP3 by *Staphylococcus aureus* in keratinocytes reduces *Staphylococcus aureus*-mediated IL-1ß and IL-17C induction

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Staphylococcus aureus is one of the major skin pathogens causing various skin infections. Infection of keratinocytes with *S. aureus* induces the expression of several cytokines including IL-1beta and IL-17C. We observed that *S. aureus* infection in primary keratinocytes led also to the upregulation of the regulatory molecule TNFAIP3 ('tumor necrosis factor alpha induced protein 3', A20). TNFAIP3 was originally identified as TNFalpha-induced protein that is able to protect cells against TNFalpha-induced apoptosis. It is known that TNFAIP3 is involved in regulatory processes of inflammation via the inhibition of NF-kappaB-activation. To evaluate a potential role of the TNFAIP3 induction by *S. aureus* in keratinocytes on cytokine expression we used TNFAIP3-specific siRNA to downregulate TNFAIP3 expression in keratinocytes. This led to increased basal levels of IL-17C and IL-1beta expression. In addition, IL-17C and IL-1beta expression was strongly induced in *S. aureus*-infected

keratinocytes treated with TNFAIP3 siRNA as compared to control siRNA-treated cells. This indicates that TNFAIP3 inhibits IL-1beta and IL-17C induction in keratinocytes probably to avoid exaggerated inflammatory reaction. To confirm the regulatory influence of TNFAIP3 on the expression of IL-17C and IL-1beta we overexpressed TNFAIP3 in primary keratinocytes. Increased TNFAIP3 expression led to a decreased basal expression of IL-17C and IL-1beta. In addition, the *S. aureus*-mediated induction of IL-17C and IL-1beta in keratinocytes was less pronounced as compared to the cells not overexpressing TNFAIP3.
Our results highlight a new strategy of *S. aureus* to subvert innate cutaneous defense by the induction of the inhibitory molecule TNFAIP3 in keratinocytes leading to decreased expression of defense molecules such as cytokines. This may offer a survival and growth advantage of *S. aureus* and may thus contribute to establish and spread the infection.

P067

Identification of chemokine profile associated to melanoma metastasis

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Chemokines play a role in metastasis by contributing to processes such angiogenesis, lymphangiogenesis, tumor immunology or tumor cell migration. The comparison of normal skin to primary tumor and metastatic to tumor-free lymph node in a spontaneously metastasizing xenotransplantation melanoma mouse model, identified twelve differently regulated chemokines. Quantitative assessment of the mRNA levels from these chemokines in T1 to T4 melanoma human samples, established a pattern of clustered chemokines. Overexpression of a highly upregulated chemokine in T4 compared to T1 in a metastatic melanoma cell line and subsequent injection into the skin of CB17-SCID mice, lead to a more invasive tumor phenotype as observed macroscopically. Histological analysis reflected the invasiveness of the surrounded tissue. A marked increase in neutrophil infiltration, lymph node weight and lung metastasis was observed. The proved lymphangiogenic capacity *in vitro* along with a favored neutrophil-tumor cell interaction could potentially explain the increment in metastasis. A positive correlation of our results in human material confirm the relevant and versatile role of chemokines during the metastatic process.

P068

Extracellular control of growth factor signalling by fibrillin microfibrils

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The dermal elastic fiber network does not only confer essential biomechanical properties such as flexibility and extensibility to the skin but also serves as an important functional scaffold for maintaining skin homeostasis. This is illustrated by mutations in essential elastic fiber building blocks, the fibrillins (fibrillin-1 and -2), leading to congenital disorders characterised by skin phenotypes ranging from thick skin in Weill-Marchesani Syndrome to thin skin in Congenital Contractural Arachnodactyly, from stiff skin in Stiff Skin Syndrome to hyperelastic and fragile skin in Marfan and Shprintzen Goldberg Syndrome. So far it is unclear how fibrillins exert control over the growth and differentiation processes resulting in this spectrum of phenotypes. The most plausible explanation is that fibrillins modulate growth factor signalling essential for proper growth and homeostasis in the skin. Recently, we have shown that fibrillin microfibrils, the tissue form of fibrillins, target and sequester growth factors of the TGF-ß superfamily. We therefore imagine fibrillin microfibrils as integration platforms for TGF-ß and BMP signaling.
By introducing dominant negative effects on fibrillin microfibrils *in vivo* we want to test this working model in a mouse model (GT8) of progressive skin fragility. GT8 mice express a truncated version of fibrillin-1 which interferes with the normal assembly process of fibrillin-1 microfibrils. Analyzing GT8 skin revealed that the fibrillin microfibrillar network looks intact within the first week of postnatal life, however at 1 week of age microfibrils appear to be fragmented. Our generated data led us to the hypothesis that upon fibrillin microfibril fragmentation sequestered growth factors are released and aberrantly activated. This leads to activation of signaling pathways resulting in the upregulation of matrix metalloproteinases. Together our data may represent a new paradigm of how skin homeostasis is maintained via the elastic fiber network.

Clinical Research

P069

The single-chain anti-TNF-alpha antibody DLX105 induces a clinical response in psoriasis patients when administered intradermally

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While systemically administered anti-TNF-alpha antibodies are effective in treating chronic plaque psoriasis, it is not clear whether these drugs need to act systemically, within the secondary lymphatic tissues, or whether inhibition of skin-produced TNF-alpha would be sufficient to mediate a clinical response. To answer this question, we conducted a clinical trial in psoriatic patients and administered the novel single chain antibody DLX105 intradermally directly into psoriatic plaques. Each patient received, on four occasions within 10 days, one injection of DLX105 into 4 cm2 of one plaque and one injection of placebo into another plaque. Ten patients received a low dose of DLX105 (20 μg in 100 μl) and another ten patients a high dose (1 mg in 100 μl). At day 14, the higher dose of DLX105 induced a mean local PASI decrease of 33% over baseline, while the placebo response was 12% ($P = 0.002$). The clinical response was accompanied by changes in biomarker expression such as K16, Ki67 and epidermal thickness as well as mRNA expression of cytokines such as IL-17, TNF-alpha, IL-12 and IFN-gamma. In summary, this study demonstrates that local inhibition of TNF-alpha is sufficient to mediate a clinical response in psoriasis patients.

P070

The role of micro RNA 181a in cutaneous squamous cell carcinoma

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Pilot experiments show low abundance of miR-181a (among others) in squamous cell carcinoma. Due to the loss of its negative regulatory capacity caused by its lowered expression, functional properties such as cellular proliferation or migration might be elevated, explaining the cancerous behavior of these cells. Preliminary data confirm our hypothesis, because artificial upregulation of this particular micro RNA leads to significant reduction in cellular proliferation. Inversely, miR-181a downregulation in HaCaT cells (a cell line representing normal human keratinocytes) provokes increased proliferation, mimicking cancerous behavior at least in part. To identify the cellular mechanisms forming the basis of this phenomenon, a micro array should display changes in several mRNA expression levels. Several knockdown approaches followed by *in vivo* testing will confirm the relevant downstream targets of miR-181a and may consequentially unravel its role in squamous cell carcinoma.

P071

SSc-overlap syndromes: a distinct clinical subgroup with significant differences in disease progression compared to lSSc and dSSc patients. Data of the German Network for Systemic Scleroderma (DNSS)

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Background: SSc-Overlap syndromes are a very heterogeneous and remarkable subgroup of SSc-patients, who present at least two connective tissue diseases (CTDs) at the same time, usually with a specific autoantibody status.
Objectives: To determine whether patients, classified as SSc-overlap syndromes, represent a distinct SSc subgroup with a disease course different from patients with limited (lSSc) and diffuse cutaneous SSc (dSSc).
Methods: The data of 3240 prospectively included patients, registered in the database of the German network for systemic sclerosis and followed between 2003 and 2013, were analyzed. The following statistical methods were used: Kaplan-Meier analysis, logistic regression and 2test.
Results: Among 3240 registered patients, 10% (325/3240) were diagnosed as SSc-overlap syndrome. Of these, 82.5% (268/325) were female with a mean age of 49.2 1.2 years and carried significantly more often other antibodies (71.1%; $P < 0.0001$), including U1RNP- (33.5%), PmScl- (16.9%), Ro-(24.7%), La- (11.0%), as well as Jo-1- (4.1%) and Ku-antibodies (3.8%).
These patients developed musculoskeletal involvement significantly earlier and more often, than patients diagnosed as lSSc and dSSc (37.8%, 47.8%; $P < 0.0001$). The onset of lung fibrosis and heart involvement in SSc-Overlap patients was significantly more frequent and earlier than in patients with limited SSc and occurred later in patients with dcSSc. Oesophagus, kidney and PAH progression was similar to lcSSc patients, whereas dcSSc patients had a significantly earlier onset. Patients with SSc-overlap syndromes were significantly more frequently treated with corticosteroids and immunosuppressive agents than other SSc subsets. Additionally, this specific subset also had a significantly lower mRSS compared to dcSSc patients (6.7 0.4 vs 15.8 0.3; $P < 0.0001$), but a very similar mean mRSS to lcSSc patients (7.2 0.2).
Conclusions: These data support the concept, that SSc-overlap syndromes should be regarded as a separate SSc subset, distinct from lSSc and dSSc.

P072

Canakinumab treatment in Schnitzler's syndrome: a multi-center randomized placebo-controlled 4-month study

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Background: Schnitzler's syndrome (SchS) is an adult-onset autoinflammatory disease characterized by urticarial exanthema and monoclonal gammopathy in combination with episodes of fever, arthralgia, fatigue, and bone and muscle pain. Anti-IL-1 targeting therapies in small patient numbers including an open-label study with canakinumab (CAN) showed to be effective in reducing the clinical symptoms of SchS.
Methods: The current placebo-controlled study was designed to assess the effects of the selective anti-IL-1ß humanized monoclonal antibody CAN on the clinical signs and symptoms of SchS in a larger patient cohort. A total of 20 patients with active disease enrolled in this multi-center trial. After a baseline period of up to 4 weeks, patients were randomized to receive a single CAN 150 mg or placebo s.c. injection (day 0) and were evaluated for treatment response at day 7. This initial study period was followed by a 16-week open label phase with CAN injections upon confirmed relapse of clinical symptoms. Efficacy was determined by changes in the physician's global assessment (PGA; range 0–20), a combined symptom score which includes 5 key symptoms of SchS (urticarial rash, fever, fatigue, arthralgia and myalgia), measurement of the inflammation markers C-reactive protein (CRP) and serum amyloid A (SAA) as well as changes in quality of life assessment (SF-36).
Results: CAN was highly effective ($P = 0.001$) in reducing median PGA total scores (14.0 to 2.0) within 7 days after first administration as compared to placebo treatment (15.0 to 13.0) in SchS patients. Also, significant ($P < 0.0001$–$P < 0.05$) improvements were observed for each key symptom score. Median CRP reduced from 9.3 mg/dL at baseline to 0.6 mg/dL at day 7 in the CAN group versus increase from 3.0 mg/dL to 5.0 mg/dL for the placebo group. Similarly, median SAA levels reduced from 428 mg/L to 13 mg/L for the CAN group versus increase from 160 mg/L to 205 mg/L for the placebo group. The median change from baseline between treatment groups for CRP ($P = 0.004$) and SAA ($P = 0.002$) was significant. Likewise, quality of life as measured by SF-36 significantly improved ($P = 0.001$) for the CAN versus placebo groups at day 7. These improvements were maintained during the 16-week open label phase of the study. A total of 22 adverse events (AEs) were reported during the study including 3 serious AEs (2 hypertensive episodes in 1 patient and severe lumbago in another patient).
Conclusion: In this 4-month study, CAN s.c. injections significantly improved the clinical signs and symptoms of SchS, reduced inflammation markers, and enhanced quality of life. CAN treatment may be considered a promising therapeutic option in these patients.

P073

Highly efficient and compatible hypertolerant shampoo

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Introduction and objectives: In the present study, we investigated the efficacy and compatibility of a rinse-off hypertolerant shampoo, which was specifically developed for hypersensitive and problematic scalp, in patients after hair transplant. The shampoo contains an extremely mild surfactant system as well as bisabolol but is free of perfumes, silicones, colorants, parabens, paraffins and betaine.

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Materials and methods: 45 subjects (42 male and 3 female), ranging from 20 to 65 years of age, were qualified for study participation after previous hair transplant at the Moser Medical Clinic in Vienna. The volunteers started to use the hypertolerant shampoo two days after the operation and continued until stitches were removed (between 7 to 23 days). They were supposed to use a realistic amount of shampoo and to wash their scalp at least once per day. No other treatment was allowed. Scalp condition and compatibility were analyzed by the plastic surgeon directly after the hair transplant as well as after stitch removal for each volunteer. The volunteers completed self assessment questionnaires to rate product efficacy and scalp compatibility at the time of stitch removal.
Results: The results of this study showed an excellent skin compatibility and product efficacy of the hypertolerant shampoo after hair transplant. This was confirmed in the dermatological scalp examinations by the plastic surgeon as well as in the volunteer's self assessment. The plastic surgeon fully recommended the further use of the shampoo. Moreover, a significant reduction in the extent of scabs and erythema was detectable by comparing the dermatological findings of the plastic surgeon directly after hair transplant to the removing of the stitches. In the self assessment, the volunteers significantly confirmed the reduction of itching, a scalp calming effect, good product mildness and cleansing performance as well as good hair care effects after shampoo treatment.
Conclusions: The hypertolerant shampoo is ideally suited for the use after hair transplant and might be therefore also useful for other critical post operative treatments. The excellent skin compatibility is based on the mild surfactant system, the calming ingredient bisabolol and the abstinence of potentially irritating ingredients.

P074

Differential influence of vemurafenib and dabrafenib on patient lymphocytes despite similar clinical efficacy in melanoma

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Background: Since the majority of melanomas eventually become resistant and progress, combining selective BRAF inhibitors (BRAFi) with immunotherapies has been proposed to achieve more durable treatment responses. Here, we explored the impact of selective BRAFi on the hosts' immune system.
Patients and methods: Clinical data, whole blood counts (WBC) and serum LDH levels of 277 vemurafenib- and 65 dabrafenib-treated melanoma patients were evaluated. Frequency and phenotype of lymphocyte subpopulations were determined by flow cytometry. CD4+ T cells were isolated by magnetic bead based separation from peripheral blood mononuclear cells (PBMC) obtained from patients before and under treatment. Cytokine secretion by CD4+ T cells was measured by multiplex assays after polyclonal stimulation.
Results: Both progression-free survival (PFS, 21 weeks for vemurafenib and dabrafenib) and overall survival (OS) (vemurafenib: 44.1 vs. dabrafenib: 46.3 weeks; $P = 0.84$) were similar in patients treated with either vemurafenib or dabrafenib. High pretreatment LDH was associated with shorter PFS and OS in both groups. During therapy, peripheral lymphocytes decreased by 24.3% (median, $P < 0.0001$) in vemurafenib-treated patients but remained unchanged in dabrafenib-treated patients (+1.2%, $P = 0.717$). Loss of lymphocytes was associated with treatment rather than disease progression as indicated by Kaplan-Meier analyses. Also, no association of decreasing lymphocytes and LDH levels prior to therapy was found. Differentiation of peripheral lymphocytes of vemurafenib-treated patients showed a significant decrease in CD4+ T cells (median change: −22.6%, $P < 0.05$). Numbers of circulating CD8+ T cells and B cells were unaffected by vemurafenib treatment while NK cell counts increased significantly during therapy ($P < 0.05$). Within CD4+ T cells obtained during treatment, an increase of CCR7+CD45RA+ (nave) and a decrease of CCR7+CD45RA- (central memory) populations was found ($P < 0.01$ for both). Furthermore, cytokine arrays detected a significant decrease in IFN-γ and IL-9 secretion ($P < 0.01$, $P < 0.05$, respectively) by CD4+ T cells obtained during treatment with vemurafenib as compared to baseline samples.
Conclusion: While both compounds have comparable clinical efficacy, vemurafenib but not dabrafenib decreases patients peripheral lymphocyte counts and alters CD4+ T cell phenotype and function. Thus, selective BRAFi can significantly affect patients' peripheral lymphocyte populations. Fully understanding these effects could be critical for successfully implementing combinatorial therapies of selective BRAF inhibitors with immunomodulatory agents.

P075

Severe alterations of body image in patients with acne inversa

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Background: Acne inversa (AI) leads chronically to disfigurement and painful eruptions in mainly intimate areas. We hypothesized an impairment of body image in AI patients.
Objectives: We studied body image in patients with AI and control subjects. Additionally, we evaluated whether disease severity and co-existing conditions (obesity, depression and anxiety) have an influence on body image.
Methods: The Frankfurt Body Concept Scale (FKKS) and the Hospital Anxiety and Depression Scale (HADS) were given to 90 voluntary study participants to assess body image in AI patients and in age-, gender- and BMI-matched controls. Moreover, correlation between the scales of FKKS, HADS and disease features was calculated.
Results: This study demonstrated for the first time that AI has a profound impact on body image. Interestingly, there was no clear correlation between severity of cutaneous alterations and extent of body image disruption. Moreover, the analyses suggest a contribution of body image alterations to depressive and anxious symptoms of affected AI patients.
Conclusions: Psychotherapeutic counselling and social support should be considered as a mean to improve body image and therefore the overall disease burden of the patients.

P076

RAS mutations in BRAF inhibitor induced skin lesions

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BRAF inhibitors represent the standard treatment of BRAF mutated metastasized melanoma. The most frequent side effects are cutaneous, including verrucous skin lesions and epithelial cancer. For epithelial cancer it is known that they frequently harbour HRAS mutations leading to tumor growth as a result of paradoxical activation of mitogen-activated protein kinase signalling.
We investigated 49 verrucous skin lesions from 15 patients which developed under BRAF inhibitor treatment morphologically and for the presence of HRAS mutations by hotspot-PCR. We found that histologically the verrucous lesions presented as vulgar verrucae ($n = 33$), squamous cell carcinomas (SCC; $n = 7$), acanthomas ($n = 4$), acantolytic (warty) dyskeratomas ($n = 2$), fibromas ($n = 2$) and a seborrhoic keratosis ($n = 1$). Of the 49 investigated samples 33 revealed a HRAS mutation (67.3%), most frequently the HRAS Q61L. Histologically most HRAS mutated samples were verrucae ($n = 27$; 81.8%) or squamous cell carcinomas ($n = 5$, 15.2%) representing 97% of histologic diagnosis in HRAS mutated samples. Hence, in addition to malignant epithelial lesions also the benign verrucous lesions reveal a HRAS mutation in the majority of cases.

P077

Deregulated type I-interferon response in TREX1-associated familial chilblain lupus

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Familial chilblain lupus is the first monogenic form of cutaneous lupus erythematosus, which in contrast to sporadic chilblain lupus manifests in early childhood. Familial chilblain lupus is caused by heterozygous mutations of genes encoding TREX1 (3repair exonuclease) or the phosphohydrolase SAMHD1.
TREX1 has a high specificity for single-stranded DNA (ssDNA). TREX1 deficiency leads to intracellular accumulation of ssDNA, which might be recognized by the innate immune system leading to a type I-interferon response that subsequently induces autoimmunity.
To investigate the potential upregulation of type I-interferons in familial chilblain lupus we analyzed skin and blood of five patients with familial chilblain lupus carrying a heterozygous mutation in TREX1 for expression of type I-interferon induced genes and proteins. In lesional skin of patients with familial chilblain lupus, high expression of the type I-interferon inducible myxovirus resistance protein A (MxA) and the chemokine CXCL10 were detected. Weak expression of MxA was also found in non lesional skin indicating chronic ongoing type I-interferon activation.
Sera of patients with familial chilblain lupus contained elevated concentrations of CXCL10 compared to sera from patients with sporadic chilblain lupus or healthy controls. In addition, mRNA levels of type I-interferon induced genes were upregulated in blood of patients with familial compared to sporadic chilblain lupus and healthy controls.
From these data we hypothesized that the stronger expression of type I-interferon induced genes might reflect a more enhanced phenotype. Indeed cutaneous involvement measured by CLASI was stronger in patients with TREX1-associated familial chilblain lupus compared to sporadic chilblain lupus. These observations document involvement of type I-interferons in the pathogenesis of familial chilblain lupus and implicate the type I-interferon signaling pathway as a potential therapeutic target in familial chilblain lupus.

P078

The cutaneous effects of afamelanotide, a novel synthetic alpha-MSH-analogue, in two clinical phase III trials for the treatment of erythropoietic protoporphyria

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Afamelanotide (Scenesse®) is a novel synthetic analogue of alpha-melanocyte-stimulating hormone [Nle4, D-Phe7] that binds to the melanocortin-1 receptor, thereby inducing melanin production in melanocytes. The subsequent increase in skin pigmentation, together with the drugs anti-inflammatory properties, offers the possibility of therapeutic benefit for distinct photodermatoses. Here, we report on the cutaneous effects of afamelanotide in the hereditary photosensitivity disorder erythropoietic protoporphyria (EPP). Three European porphyria expert centers recruited 41 patients with EPP for the treatment with afamelanotide in two prospective, double-blinded, placebo-controlled, multicenter phase III trials and during a subsequent compassionate use period (supported by Clinuvel Pharmaceuticals Ltd, Melbourne, Vic., Australia). All individuals received either the active drug or the placebo every 2 months by means of a slow-releasing subcutaneous implant. The general cutaneous effects observed under this treatment comprisedincreased skin pigmentation in melanocytic nevi and lentigines located in sun-exposed areas of the body as well as an increased pigmentation in non-sun-exposed body sites. In single individuals, we observed pronounced perioral and localized labial pigmentation, arachnoid-shaped hyperpigmentation at the implantation site and linear postinflammatory hyperpigmentation. Additionally, some patients reported facial flushing shortly after implantation. Importantly, afamelanotide appeared to be effective in amelioration of the acute, burning and painful photosensitivity commonly experienced by EPP patients, although formal data analysis has not yet been reported. No major side effects were observed. In conclusion, our data revealed significant cutaneous responses to this novel drug, which were generally acceptable to patients and would be of sufficient magnitude to explain a therapeutic response in EPP. We strongly believe that a wider benefit for this drug could emerge in other photosensitivity disorders.

P079

High-dose intravenous immunoglobulins – a therapeutic option in recalcitrant erosive oral lichen planus?

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We here present three female patients with severe erosive oral lichen planus who showed a refractory course of disease despite various topical and systemic treatments. In light of the proven efficacy of intravenous immunoglobulins in chronic inflammatory skin disorders, all the three patients received a combination of IVIG (2 g /kg/month) and 0.5 mg acitretin/kg/day for at least 7 months. Clinical improvement of mucosal lesions was measured by the Autoimmune Bullous Skin Disorder Intensity Score (ABSIS 1) and the functional sequelae by a subjective score (ABSIS 2). All the patients showed variable effects of IVIG treatment. Patient RT did not show a significant improvement after 6 monthly cycles of IVIG by ABSIS 1 (mo 0: 15, mo 6: 10, mo 15: 7) but showed a significant decrease of subjective symptoms determined by ABSIS 2 (mo 0: 4, mo 6: 0, mo 15: 0). Patients MH and UJ also showed a long-term improvement of the subjective symptoms (ABSIS 2; mo 0: 19, mo 6: 10, mo 15: 6) while the objective disease activity was not consistently improved (ABSIS 1; mo 0: 9.5, mo 6: 5, mo 15: 10). Patient MH received only two cycles of IVIG due to treatment-induced leukopenia but showed a complete clearance of mucosal lesions at moths 6 and 8. In summary, treatment with high-dose IVIG should be considered as a therapeutic option in otherwise recalcitrant erosive oral lichen planus.

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Dermato-Endocrinology

P080

The xenobiotic receptor pregnane X receptor modulates migration and carcinogen metabolism in Langerhans cells

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Skin exposure to haptens, microbes or chemicals including carcinogens induces migration of skin DC. The pregnane X receptor (PXR) is a transcription factor activated by and regulating the metabolism of xenobiotics, hormones and cholesterol metabolites. We here found that PXR is expressed in different subsets of mouse and human immature dendritic cells (DC) especially Langerhans cells (LC) where it controls CCR7 expression via TGF-β. In LC, PXR signals via the TGF-β pathway by preventing the down-regulation of active form of smad2/3 that normally occurs upon LC maturation. *In vivo*, PXR deficiency increases migration of LC after skin exposure to a hapten (TNCB) but also to a carcinogen (DMBA), suggesting an activated skin immune response. Furthermore, PXR is dramatically decreased in CCR7+ cells in squamous cell carcinoma (SCC) and PXR deficiency delays the appearance of chemically-induced papillomas and SCC in a two-stage model of chemical carcinogenesis. Moreover, PXR deficiency protects against DNA damage as measured by levels of phospho-H2AX. Redundant functions and cross-talk exist between PXR and Ahr in the metabolism of chemicals and carcinogens and both the PXR and Ahr have been shown to be involved in the development of SCC via the regulation of different enzymes, CYP1 and CYP3A respectively. We here show that expression of PXR and Cyp3a11 is induced as early as 1 h after topical application of DMBA and continuously increases with time, as opposed to Ahr expression which remains unchanged. Interestingly, Cyp1b1 and Cyp1a1 expression exhibits a bell curve peaking at 4 and 24 h, respectively. In summary, PXR might be involved in the metabolism of chemicals and thus in cancer processes in the skin, rendering PXR an attractive target for future anti-cancer drug development.

P081

Co-culture of skin explants with SZ95 sebocytes: friends with benefits

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In our previous work we have presented the development of a co-culture skin explant model with SZ95 sebocytes in direct contact. Furthermore, we have proven with morphological analysis (hematoxylin eosin staining) and functional assays (Ki67, TUNEL, IL-6) that this co-culture setting is beneficial for skin viability and homeostasis and it also induces a more *in vivo*-like phenotype of SZ95 sebocytes. Co-culture of skin explants with fibroblasts as a control cell type did not improve the morphology of the skin explant epidermis and did not decrease IL-6 secretion in the supernatant, suggesting a sebocyte-specific type of the results. Moreover, co-culture of skin explants with SZ95 sebocytes in humoral contact did not improve skin morphology *ex vivo*. Time course experiments of skin explants with SZ95 sebocytes in direct contact showed a benefit of the skin explant structural integrity already from the second day of the co-culture, with concomitant stronger expression of the epithelial membrane antigen from SZ95 sebocytes, in comparison to the control. The aforementioned results elucidate the importance of sebocytes for *ex vivo* skin homeostasis and corroborate the addition of SZ95 sebocytes to future 3D models.

P082

Anti-inflammatory and anti-pruritic activity of an alkylamide from *Echinacea purpurea* in murine models of delayed-type hypersensitivity and atopic dermatitis

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Extracts from Echinacea purpurea (purple cornflower) are known to have immunomodulatory effects. Alkamides are the major lipophilic constituents in the extracts of Echinacea roots. Due to their structural similarity to anandamide several of these alkamides were found to be ligands of cannabinoid receptors 1 and 2 (CB1-R and CB2-R). These alkamides were shown to inhibit prostaglandin E production following LPS challenge *in vitro*. *N*-Isobutyl dodeca-2E,4E-diene amide binds to CB2-R with Ki = 60 nM and to CB1-R with Ki = 1.94 M. Moreover, it also significantly inhibited LPS-induced TNF-α, IL-1β, and IL-12p70 expression *in vitro* in a CB2-independent manner. Since the endocannabinoid system plays an important role in inflammatory disorders and is also expressed in skin, we were interested in the anti-inflammatory and anti-pruritic activity of *N*-isobutyl dodeca-2E,4E-diene amide in mouse models of inflammatory and pruritic skin diseases.

The efficacy of topical *N*-isobutyl dodeca-2E,4E-diene amide was assessed in a mouse model of delayed-type hypersensitivity regarding amelioration of itching and inflammation. Mice (n = 5) were sensitized with oxazolone on day 0, challenged on days 7, 9, 11, 14, 16 and 18 and treated daily from day 14 until day 18 with the alkamide (1% (w/v) in 100 L acetone) or betamethasone dipropionate (0.05% (w/v) in 100 L acetone) as positive control. On day 18 both treatments did reduce scratching significantly ($P < 0.05$) not only in the early phase after the challenge with oxazolone but over a period of 22 h. Histological analysis (H&E) of treated ears (n = 5) showed effects on ear thickness, epidermal thickness and dermal infiltrate.

In addition, *N*-isobutyl dodeca-2E,4E-diene amide was tested in NC/Nga mice, an inbred mouse strain that develops a spontaneous AD-like pathology in non-sterile housing conditions. Mice (n = 9) were exposed to dust mite antigen and randomized prior to the start of the study on the basis of the average total clinical disease score (approximately 1) consisting of erythema (0–3), edema or papulations (0–3) and oozing, crusts or hemorrhages (0–3). Mice received a topical dose of vehicle (20 L, acetone/olive oil (4:1)) or *N*-isobutyl dodeca-2E,4E-diene amide (20 L, 1% (w/v) in acetone/olive oil (4:1)) that was applied once daily to the back for 30 days. 0.1% tacrolimus ointment (10 mg/cm) was used as positive control. Clinical score was assessed twice weekly. In non-treated mice an increase of the total clinical score of 1.9 was observed from day 1 to day 30. The further development of the disease was significantly ($P < 0.01$) attenuated by treatment with *N*-isobutyl dodeca-2E,4E-diene amide. The effect was comparable to the one observed for tacrolimus (0.1%).

Topical application of an alkamide from Echinacea purpurea showed significant anti-inflammatory and antipruritic effects in two dermatitis models. Thus, this substance class should be explored in detail as potential therapeutic option for the topical treatment of cutaneous inflammatory diseases like atopic dermatitis.

P083

Insulin resistance in microvascular dermal endothelial cells contributes to the pathogenesis of psoriasis and its co-morbidities

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There is growing evidence that psoriasis, a chronic inflammatory skin disease, has a considerable systemic dimension as it is associated with severe co-morbidities such as the metabolic syndrome, diabetes and cardiovascular diseases. In addition, there are striking similarities between a psoriatic and an artherosclerotic plaque, which make us hypothesize that a common pathomechanism is underlying the skin symptoms as well as the changes at the endothelial wall. A possible link might be represented by inflammation-driven insulin resistance, which was shown to contribute to the psoriatic plaque formation. This hypothesis is supported by the fact that under healthy conditions insulin is both cardio protective and anti-inflammatory.

We could previously show that pro-inflammtory cytokines (IL-1β, IL-17, IL-22, IL-23 and TNF-α) which are involved in the pathogenesis of psoriasis can induce insulin resistance in primary dermal microvascular endothelial cells. This was measured by the insulin-dependent phosphorylation of PKB, which is reduced in the presence of pro-inflammatory cytokines. By using chemical inhibitors as well as siRNA mediated knockdown, we could demonstrate that JNK is the key kinase that mediates insulin resistance.

In order to investigate the functional consequences of insulin resistance, we examined the expression of adhesion molecules, which are involved in the process of transendothelial migration of lymphocytes. The expression of these molecules on the cell surface is repressed by insulin and remains high under conditions of insulin resistance. Furthermore we investigated the migratory and adhesive characteristics of T-cells on the endothelial cell layer under insulin resistant conditions.

We could show by a static approach that the adhesion of T-cells to endothelial cells is increased under insulin resistant conditions and can not be repressed by the addition of insulin. Under flow conditions, we could see that also the rolling and migratory behaviour of the T-cells is shifted towards a pathological situation.

In summary, we suggest that the systemic inflammation as in psoriasis leads to a disturbed insulin response at the endothelial wall. This contributes to the pathogenesis of psoriasis and its co-morbidities by regulating the presentation of adhesion molecules on the endothelial surface and thereby contributing to lymphocyte extravasation into the skin. Therapeutic approaches interfering with the altered insulin response in psoriasis might by very effective by targeting both the dermal as well as the cardiovascular dimension of psoriasis.

P084

Epidermal insulin resistance as a therapeutic target in *Acanthosis nigricans*?

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There is increasing evidence that insulin plays a role in skin homeostasis and that epidermal insulin resistance contributes to different skin pathologies such as psoriasis or diabetes associated skin conditions like acanthosis nigricans (AN). However, details of the molecular pathomechanisms as well as differences between the distinct entities have not yet been fully established.

We therefore studied the changes in epidermal insulin signaling under optimized anti-diabetic treatment in a patient suffering from AN and type II diabetes. Skin biopsies before therapy showed signs of epidermal insulin resistance as marked by enhanced inhibitory phosphorylation of IRS-1 and reduced Akt/PKB activation which both transmit signals from the insulin receptor. This inhibition seems to be mediated by mTOR as IRS-1 is phosphorylated at the mTOR phosphorylation site serine 636/9 and strong activation of the mTOR kinase was found as well.

A drop of the HBA1C from 6.3 to 5.7 under optimized anti-diabetic therapy with the GLP-1 analogon liraglutide was paralleled by a clinical improvement of the acanthosis nigricans. Interestingly, signs of epidermal insulin resistance ameliorated during anti-diabetic treatment in non-lesional skin, while signs of epidermal insulin resistance remained in lesional skin.

Thus, our data suggests a putative role for mTOR in diabetes-associated AN. Diabetes-related mTOR hyperactivation seems not only to render metabolic tissues unable to respond to insulin properly, but also disturbs insulin signaling in keratinocytes, which then interferes with skin homeostasis. Therefore our example suggests the exploration of the pharmacologically well-established mTOR inhibitor rapamycin/sirolimus as a therapeutic option for AN.

P085

Impact of topical glucocorticoids on aromatase activity tested within a full-thickness skin model

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Aromatase is a member of the P450 enzyme family. It is expressed in a variety of tissues and cell species e.g. in liver, skin and adipose tissue. The enzyme is located in the membrane of the smooth endoplasmatic reticulum and catalyzes two reactions in the process of estrogen biosynthesis from androgens. Specifically, the aromatase converts androstendione to estrone and testosterone to estradiol.

To produce three-dimensional full-thickness skin models (FTSMs), primary human fibroblasts were seeded onto a washed and equilibrated collagen matrices. After a 2 weeks cultivation period primary human keratinocytes were seeded on top of the scaffolds. The models were cultivated for another week under submersed conditions before they were lifted to the air-liquid interface (ALI) and cultured until they become sufficiently stratified.

In our experimental setup different corticoid creams (Dermatop®, Ecural®, base cream DAC each with 2.5% hydrocortisone and 0.1% betamethasone-17-valerate) and the corresponding base creams (Dermatop base cream®, Essex base cream® and base cream DAC) were applied on epidermal top of the skin equivalents using a Transferpettor®.

In order to analyze the aromatase activity using the Roche Elecsys® 2010 systems, 20 M testosterone were added as substrate for the enzyme to the ALI medium of FTSM replicates. The estradiol concentration in the cell free culture medium was measured as analytical parameter of aromatase activity after 2, 4 and 7 days.

Furthermore, histological samples were obtained.

Our results demonstrate that the aromatase activity in three-dimensional organotypic skin models is stimulated by all applied corticoid creams in a time-dependent manner. The highest estradiol concentrations are obtained by treatment with Dermatop® and base cream DAC containing 2.5% hydrocortisone.

Interestingly, application of the corresponding base creams without active compound also increases the measured estradiol concentration.

These data strongly indicate that one or more ingredients of the base creams induce aromatase activity in skin cells. Consequently, we intent to investigate the effects of these ingredients on aromatase activity in both monolayer cultures and FTSMs.

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P086

Sebaceous gland-specific gene targeting in mice

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Sebaceous glands (SG) elicit a variety of functions in the skin, including maintenance of the epidermal barrier and hair follicle integrity. SG-associated pathologies include the most common skin disease, acne, and benign and malignant tumors, among others. Up to now, regulatory sequences of keratin genes or other structural proteins have been used for targeting genes in the epithelial compartment of the skin. While effective, this strategy has the drawback that numerous cell types in the epidermis and in the pilosebaceous unit are targeted in parallel, potentially causing side effects and unspecific phenotypes.

To establish a mouse line with SG-specific expression of cre recombinase, we replaced the first exon of Scd3, a gene encoding an enzyme of the Stearoyl-coenzyme A desaturase family that is expressed exclusively in sebocytes, with the cDNA for codon-improved cre recombinase (iCre) via homologous recombination in embryonic stem cells. After obtaining germline transmission of the modified allele via chimeric mice, we crossed the positive offspring to the Rosa26-LacZ reporter line. Recombination of the reporter locus, examined by histochemical detection of β-galactosidase (β-gal) in animals heterozygous for both alleles, confirmed that cre activity in both back and tail skin was limited to the SG, with no staining in the epidermis, dermis, or hair follicle. As expected, β-gal staining was also evident in free SGs (Meibomian gland and preputial gland). We additionally detected a few β-gal-positive cells in the intestinal epithelium and in the cerebral cortex, indicating that the expression of Scd3 may not be fully restricted to the skin. Thus, Scd3-iCre mice can be used to target genes specifically in the SG.

We believe that this new mouse line will become a useful tool for studying the roles of the SG in health and disease.

P087

UV-induced impairment of differentiation homeostasis in human skin is prevented by melatonin

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Human skin, the largest organ of the body and barrier to the environment, plays a crucial role in the regulation of whole-body homeostasis including the mechanical (physical barrier) and functional (immune and antioxidative system, pigmentation) defense against life-long exposure to environmental stressors. Melatonin (*N*-acetyl-5-methoxytryptamine) and its main kynurenic metabolite AFMK (N1-acetyl-N2-formyl-5-methoxykynuramine) have recently been shown to significantly enhance epidermal differentiation of human *ex vivo* skin, therein contributing to maintaining skin homeostasis and barrier. One main external stressor that impairs skin homeostasis and barrier function is ultraviolet radiation (UVR), and melatonin has earlier been shown to be one of the most potent protective agents to counteract UVR-induced oxidative damage by building the melatoninergic antioxidative system of the skin. Here, we investigate the ability of melatonin as a regulator of skin proliferation and differentiation under increasing UVR-doses (100, 300 mJ/cm UVB/A) in human *ex vivo* full-thickness skin organ culture. Key markers of non-differentiating (proliferating) basal layer keratinocytes (cytokeratin-14; K14) and keratinocytes of the differentiating spinosum (cytokeratin-10; K10) and granulosum (involucrin; IVL) layers have been investigated for their response to UVR and parallel incubation with melatonin in comparison to non-melatonin treated controls. Experiments were conducted in a UV-dose- (0, 100, 300 mJ/cm2) and time-dependent manner (0, 24, 48 h post-UVR). Immunofluorescence labeling showed a significant down-regulation of K14-positive basal layer keratinocytes by 38% as well as K10- and IVL-positivity by 35% and 39%, respectively, directly after irradiation (0 h post-UVR) at the dose of 100 mJ/cm2 compared to sham-irradiated skin ($P < 0.001$). Decrease of these markers was stronger at the dose of 300 mJ/cm2. Pre-incubation with melatonin (10^{-3} M, 1 h) significantly enhanced expression of K14 at 0 h post-UVR by 29% (100 mJ/cm2) and 48% (300 mJ/cm2), K10 by 20% (100 mJ/cm2) and 41% (300 mJ/cm2), and IVL by 31% (100 mJ/cm2) and 52% (300 mJ/cm2). Interestingly, non-irradiated skin, cultured over 24 h and 48 h showed significantly enhanced K14-, K10- and IVL-positive keratinocytes indicative for the physiological differentiation process, which was also significantly enhanced by melatonin ranging from 12 to 18% ($P < 0.001$). These observations in combination with our previous reports confirm that melatonin enhances skin proliferation and differentiation not only in physiologic conditions, but also in pathologic conditions of UVR exposure. To conclude, melatonin is able to maintain structure and integrity of human epidermis and therefore can supposedly prevent disturbances of the skin barrier in physiologic and UVR stress-mediated conditions in human skin.

P088

Alpha-melanocyte-stimulating hormone orchestrates an active anti-inflammatory activation pattern in human basophils of allergic rhinitis patients and controls

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Background: α-Melanocyte-stimulating hormone (α-MSH) was shown to exert antiallergic effects in human basophils.

Objective: This study aims to extend our current knowledge about the effects of α-MSH on basophils especially from patients with allergic rhinitis (AR).

Methods: We included three different study groups. In the first group we analyzed nasal tissue of AR patients and healthy controls ($n = 9$ each), in the second group whole blood basophils of AR patients ($n = 14$) and healthy controls ($n = 19$). In the third group we investigated isolated basophils derived from healthy controls ($n = 13$). Tissue expression before and after nasal allergen provocation and *in vitro* inducibility of melanocortin receptor-1 (MC-1R) was analyzed using immunofluorescent staining, flow cytometry and qRT-PCR. Basophil activity was evaluated by measurement of CD203c, cytokine release and chemotaxis. Viability was analysed by means of propidiumiodid/AnnexinV and Jc-1 staining.

Results: MC-1R positive basophils were increased in nasal mucosa tissue of AR patients 24 h after nasal allergen challenge. MC-1R expression was significantly higher in blood basophils of patients with AR compared to healthy controls. MC-1R expression was inducible on whole blood basophils by different stimuli including fMLP and anti-IgE. -MSH inhibited anti-IgE and grass pollen induced upregulation of the clinically relevant activation marker CD203c. α-MSH functioned as a chemoattractant and increased viability in basophils in isolated basophils. Moreover, α-MSH induced the release of the anti-inflammatory cytokine IL-10.

Conclusion: In AR nasal allergen provocation enhances influx of MC-1R positive basophils. Additionally, α-MSH triggers an anti-inflammatory activation pattern in basophils with inhibition of CD203c and release of IL-10.

P089

Thyroid hormones regulate key parameters of mitochondrial biology in human skin epithelium *in situ* and may exert anti-aging effects

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Thyroid hormones (THs) strongly regulate mitochondrial biology, and thyroid dysfunction often correlates with skin abnormalities. Here we investigated whether THs affect mitochondrial biology and aging in human skin and, given the mitochondrial theory of aging, whether the two events are correlated. Organ-cultured human skin or cultured epidermal keratinocytes were treated with triiodothyronine (T3) 100 pM or thyroxine (T4) 100 nM and/or potassium cyanide (KCN) (abrogates mitochondrial respiration) 300 M for 24 h or 6 days.

Treatment with T3 or T4 for 24 h up-regulated the immoreactivity (IR) of the mitochondrially-encoded cytochrome c oxidase I (MTCO1) and of the mitochondrial transcription factor A (TFAM) and increased the activity of complexes I. Moreover TH treatment increased mitochondrial biogenesis, as shown by the increased number of perinuclear mitochondria in epidermal keratinocytes (TEM).

Next, we asked whether THs exert beneficial effects on human skin aging, analyzing some standard read-out parameters that are up-regulated (MMP1, MMP2 and MMP9) or down-regulated (fibrillin 1, collagen I and III) in aged skin and ROS production. Interestingly in organ cultured skin, MMP1 IR decreased and fibrillin 1 fibers appeared less fragmented after 24 h of treatment with T3 and T4, and MMP -2 and -9 activity decreased after 6 days of treatment with THs. Furthermore, collagen I and III IR was up regulated after 6 days of treatment with T3. Finally ROS production was decreased in cultured ORS keratinocytes after 24 h of treatment with T3.

In order to check, whether these potential anti-aging effects of THs depend on intact mitochondrial function, oxidative phosphorylation was blocked with KCN for 24 h. In the presence of KCN, MMP1 IR did not decrease after 24 h of treatment with T3 or T4 suggesting a possible relationship between the anti-aging and mitochondrial effects of THs.

These data provide the first evidence that THs are strong endocrine stimulators of mitochondrial activity and biogenesis in human epidermis *in situ* and raise the possibility that epidermal aging may be counteracted by (topically applicable?) THs in a mitochondrial function-dependent manner.

P090

Characterization of the lipid droplet proteome of a sebaceous gland cell line

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Intracellular lipid accumulation and storage is accomplished by lipid droplets (LD), cytoplasmic structures found in the cells of a variety of organisms from bacteria to mammals. Structurally, LDs are formed by a hydrophobic neutral lipid core surrounded by a phospholipid monolayer in which numerous proteins are embedded. Functionally, rather than being inert structures, LD and LD-associated proteins have been associated with a number of processes, including lipid metabolism, signal transduction, and protein storage. While LDs have been intensively studied in adipocytes, and the LD proteome of a variety of cells has been characterized, the LD-associated proteins of sebocytes have not been evaluated systematically so far. This is surprising, considering that lipid synthesis (sebaceous lipogenesis) is the key feature of sebocyte differentiation.

We treated SZ95 sebaceous gland cells with linoleic acid for 48 h to increase the number and the size of LDs. After cell homogenization and density gradient centrifugation, the LD fraction was collected with a tube slicer. LD proteins and the corresponding cell pellet proteins were pre-fractioned by SDS-PAGE and analysed by nano liquid chromatography and tandem mass spectrometry (LC-MS/MS), leading to the identification of 696 and 1553 proteins, respectively, at a false discovery rate <1%. The quantitative comparison of protein abundances revealed at least 60 proteins to be more than 2-fold enriched in the LD fractions.

Importantly, LD fractions contained high levels of PLIN2 and PLIN3, classical LD-associated proteins, which we have previously identified as the most abundant perilipin family members in SZ95 sebocytes. Approximately 50% of the identified proteins have been identified previously in LDs, demonstrating the reliability of the LD protein dataset. In agreement with previous studies on the proteome of LDs isolated from other cell lines, about 80% of the proteins identified in SZ95 sebocyte LDs were assigned to lipid metabolism, membrane trafficking, or cell signalling pathways. Interestingly, we also identified a well-known antimicrobial peptide previously supposed to be expressed only in sweat glands.

This study identified several LD-associated proteins that may play a role in the physiology of sebaceous gland and possibly represent novel targets for the treatment of sebaceous gland-associated diseases.

P091

Tropisetron prevents experimentally induced lung fibrosis

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Fibrosis of the lung is a common complication in patients with systemic sclerosis (SSc). Treatment options for this condition remain limited. Thus, novel antifibrotic strategies are needed. Recently we reported that tropisetron, an approved antiemetic agent originally characterized as a serotonin (5-HT) receptor modulator, suppressed transforming growth factor-beta1-mediated collagen synthesis in normal human dermal fibroblasts (HDFs) as well as in dermal fibroblasts from patients with SSc. This effect of tropisetron was independent of the 5-HT3 and 5-HT4 receptor but mediated via the alpha7 nicotinic acetylcholine receptor (alpha7nAchR) in HDFs. Importantly tropisetron had antifibrogenic and antifibrotic effects in experimentally induced skin fibrosis of mice. The aim of this study was to test whether tropisetron has antifibrotic effects beyond the skin. First we established a mouse model in which lung fibrosis is induced by single pharyngeal aspiration of bleomycin (BLM). Tropisetron was administered for 15 days via subcutaneously implanted minipumps. As shown by real-time RT-PCR tropisetron significantly reduced collagen type I and III mRNA expression in the lungs compared with BLM-treated mice. In accordance with this protein amounts of collagen type I – as determined by pepsin digestion and SDS-PAGE – were likewise lower in lungs from mice receiving tropisetron plus BLM versus mice injected with BLM alone. Reduced lung fibrosis in tropisetron-treated mice could be confirmed by Masson Trichrom staining. To assess the relevance of these findings in the human system we performed an expression analysis of the putative tropisetron receptors in human lung fibroblasts. Neither 5-HT3 nor 5-HT4 receptors could be detected while these cells expressed the previously identified off-target receptor, alpha7nAchR. Our data show that tropisetron has antifibrogenic potential not only in the skin but also in the lung. Further studies have to define the precise role of the alpha7nAchR in collagen synthesis and fibrosis of the lung.

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P092

Beta-endorphin – an emerging antifibrotic neuropeptide

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There is increasing evidence that neuroendocrine mediators can affect the functional state of fibroblasts including collagen metabolism and tissue fibrosis. Serotonin (5-HT) was shown to directly suppress a transforming growth factor-beta1 (TGF-beta1)-mediated collagen synthesis in human dermal fibroblasts (HDFs). Pharmacologic inactivation of 5-HT(2B) receptors prevented experimentally induced skin fibrosis. Other examples of such antifibrotic neuroendocrine mediators include endocannabinoids, activators of alpha7 nicotinic acetylcholine receptors and melanocortin peptides. The latter have been shown to attenuate skin fibrosis in the bleomycin (BLM) model of scleroderma. Here we show that beta-endorphin (beta-ED), a proopiomelanocortin-derived peptide classically acting via opioid receptors (ORs), is another emerging antifibrotic neuropeptide. Beta-ED at nanomolar doses suppressed collagen type I (COL(I)) secretion in HDFs in a TGF-beta1-dependent manner. This effect occurred at protein and mRNA level of COL(I) and was independent of SMAD3 signaling. Interestingly, HDFs did express none of the classical (mu-, delta- and kappa-) ORs suggesting an OR-independent off-target effect of beta-ED. In accordance with this beta-ED failed to suppress forskolin-mediated increase of intracellular cAMP as would be predicted for an OR-mediated signaling response, i. e. activation of Gi via ORs. Moreover, beta-ED did not alter intracellular Ca^{2+} mobilization as reported in classical OR-mediated pathways. The significance of these *in vitro* findings was corroborated in the BLM model of scleroderma. Here, beta-ED significantly suppressed experimentally induced skin fibrosis as shown by quantitative COL(I) content analysis at protein and RNA level as well as by histochemistry. Our data show that beta-ED is the lead substance of a new class of neuropeptides that directly affect collagen metabolism and reduce experimentally induced fibrosis.

P093

A novel neuro-regulation of a key signaling pathway in human skin: VIP regulates intrafollicular c-Kit expression

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Since c-Kit (SCF receptor) is one of the critical growth factor receptors for various progenitor cell populations, mast cells (MC) and melanocytes, it is important to understand how its expression is regulated in normal human skin. Here, we report a novel neuro-regulatory mechanism for c-Kit expression that we came across serendipitously while assessing the effects of the immunoinhibitory sensory neuropeptide, vasoactive intestinal peptide (VIP), on c-Kit+ MCs in the mesenchyme of microdissected, organ-cultured human scalp hair follicles (HFs).

First, we noted prominent c-Kit expression not only in HF-associated MCs and melanocytes, but also in human hair matrix keratinocytes, just as we had previously reported for murine hair matrix keratinocytes. Moreover, we observed that VIP appeared to up-regulate c-Kit immunoreactivity in the human hair matrix *in situ*. Such effect of VIP on c-Kit had not been reported before, while it was known that VIP promotes proliferation of human epidermal keratinocytes *in vitro* and up-regulates its own receptors. Moreover, the VIP level is elevated in hyperproliferative psoriatic epidermal plaques. To follow up this initial observation, we treated organ-cultured human anagen scalp HFs with 3×10^{-7} M VIP for 6 h, 3 or 7 days and then ran quantitative (immuno-)histomorphomtry and qRT-PCR analyses.

This revealed a relatively selective, significant increase in c-Kit protein expression in human hair matrix, but not in the HF mesenchyme, in VIP- compared to vehicle-treated HFs. VIP-treated HFs also expressed higher mRNA transcript levels for c-Kit. Furthermore, VIP mRNA expression was significantly lowered whereas PACAP was strongly up-regulated by VIP treatment. The mRNA transcript levels of VIP receptors, VPAC1 and 2 in human HFs were also influenced by VIP treatment. Hair matrix proliferation and apoptosis (Ki-67/TUNEL) as well as HF pigmentation appeared to be relatively unchanged after 6 days of VIP treatment, even though hair shaft elongation was promoted early after VIP treatment.

Taken together these results not only suggest a new regulatory role of VIP in human HF (neuro-) biology, which we are currently exploring systematically, but also a novel neuro-regulatory signalling system. The important cutaneous neuropeptide (VIP) may increase the sensitivity of selected, rapidly proliferating epithelial cells (human hair matrix keratinocytes) of the stimulation by a key growth factor (SCF) via up-regulating the expression of its cognate receptor (c-Kit). This cross-regulation of critical signalling pathways in human skin may invite therapeutic targeting in diseases, where insufficient or excessive c-Kit expression is pathobiologically relevant.

P094

Gender-specific medicine: prevalence of dermatological disorders in hospitalized geriatric patients

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In order to study the prevalence of skin disorders among hospitalized geriatric patients in Germany, a pilot study was conducted in the Evangelisches Geriatriezentrum Berlin, a hospital in which elderly, geriatric patients with acute and chronic diseases are treated. 110 geriatric patients (60 females and 50 males) underwent a complete dermatological examination from March 2013 to August 2013. The study has been approved by the Charit Universitaetsmedizin Berlin ethics committee. The collected information was stratified according to the dermatological diagnosis, accompanying diseases and medication, age and gender of the patients. Among 73 dermatological disorders detected the top ten constituted tinea unguium, varicosis, solar lentigo, seborrheic keratoses, intertrigo, miscellaneous benign and *in situ* epithelial tumours (including actinic keratoses), decubitus, tinea pedis, seborrheic dermatitis and oral candidosis, in descending order of prevalence. Women showed a greater susceptibility to solar lentigo, varicosis, tinea unguium, seborrheic keratoses, intertrigo, miscellaneous benign and *in situ* epithelial tumours, seborrheic dermatitis, decubitus, rosacea and oral candidosis. On the other hand, the following disorders were observed in men in descending order: tinea unguium, varicosis, seborrheic keratoses, rosacea, untreated malignant epithelial tumours, solar lentigo, miscellaneous benign and *in situ* epithelial tumours, varicose eczema, seborrheic dermatitis and decubitus. Most of the patients, especially men, had not seeked for a dermatologist's expertise because of lack of understanding for the specific disorder or restricted physical abilities. Furthermore, several associations of skin diseases with systemic ones have been documented. Understanding the mechanisms of ageing and of accompanying diseases as well as their gender classification can form the basis for comprehensive, knowledge-based prevention of age-associated diseases and extension of healthy lifespan. In addition, a joint effort to raise public awareness, patients education, preventive measures and consistent monitoring of high-risk groups are of great importance.

P095

Human intestinal fibroblasts are novel target cells for alpha-melanocyte-stimulating hormone – implications for the treatment of inflammatory and fibrotic diseases of the gut with melanocortin peptides and derivatives

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Melanocortin peptides such as alpha-melanocyte-stimulating hormone (alpha-MSH) elicit their effects via melanocortin receptors (MCs). We previously demonstrated that human dermal fibroblasts express functional MC1. Treatment of these cells with alpha-MSH suppressed collagen synthesis in a transforming growth factor-beta1 (TGF-beta1)-dependent manner. *In vivo*, alpha-MSH further reduced experimentally induced skin fibrosis. Here we wondered if human intestinal fibroblasts, key effectors of fibrotic diseases of the gut, are also targets for melanocortins. Of note, alpha-MSH was previously shown to attenuate experimentally induced colitis. Mice with signaling-deficient MC1 develop aggravated intestinal inflammation after chemically induced colitis. A melanocortin tripeptide derivative of alpha-MSH, KdPT, mediated anti-inflammatory actions in a number of experimentally induced colitis models upon which phase II trials with KdPT have been launched in patients with active ulcerative colitis. Therefore, we first isolated human intestinal fibroblasts ($n = 5$) from macroscopically normal colonic specimens of patients undergoing scheduled colonic surgery. Immunocytochemistry with anti-desmin, anti-vimentin and anti-alpha-smooth muscle actin antibodies confirmed a myofibroblast phenotype of the cells. MC expression profiling revealed that these cells exclusively express MC1. Truncated transcripts for proopiomelanocortin (POMC) were also detected but no functional full-length POMC mRNA. In accordance cells lacked POMC protein expression ruling out an autocrine loop for alpha-MSH. Next, we tested if alpha-MSH induces prototypical signal transduction responses. No Ca^{2+} mobilization was detected by FURA-2AM loading, stimulation with alpha-MSH and fluorescence analysis. However, alpha-MSH at doses between 10^{-6} and 10^{-10} M significantly suppressed collagen type I secretion induced by TGF-beta1. This effect was not paralleled by reduction in corresponding COL(I) mRNAs – resembling the response of human dermal fibroblasts to alpha-MSH. Our findings demonstrate that human intestinal fibroblasts are novel targets for alpha-MSH. It will be intriguing to assess the effect of other melanocortins and derivatives in these cells on collagen metabolism as well as to investigate the impact of them in animal models of intestinal fibrosis.

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Alpha-melanocyte-stimulating hormone reduces bleomycin-mediated collagen synthesis in human dermal fibroblasts via catalase

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Neuroendocrine mediators such as melanocortins, serotonin or endocannabionoids are currently emerging as novel regulators of collagen synthesis and skin fibrosis. Accordingly, we previously reported that alpha-melanocyte-stimulating hormone (alpha-MSH) supresses bleomycin (BLM)-induced collagen synthesis in human dermal fibroblasts (HDFs) in vitro via reduction of oxidative stress. In vivo alpha-MSH further prevented experimentally induced skin fibrosis in the BLM model of scleroderma. However, the molecular mechanism behind this effect of alpha-MSH remains to be defined. We hypothesized that alpha-MSH via catalase, a master regulator in oxidative stress defense, exerts its beneficial effects against BLM-induced collagen synthesis and fibrosis. Treatment of HDFs with exogenous catalase abrogated the inductive effect of BLM on COL(I) expression at RNA and protein level. Gene knock-down of catalase by siRNA likewise neutralized the impact of BLM on COL (I) synthesis. Stimulation with alpha-MSH enhanced endogenous catalase enzyme activity of HDFs within 1 hr while having no effect on mRNA and protein expression of this enzyme. Importantly, a functional melanocortin 1 receptor (MC1) was essential for the suppressive effect of alpha-MSH on BLM-induced collagen synthesis since HDFs carrying loss of function alleles of MC1R did not react to the neuropeptide as cells carrying wild-type MC1R alleles. To finally assess the role of a functional MC1 in the context of BLM-induced collagen synthesis in vivo we injected mice with signalling-deficient MC1 (recessive yellow C57BL/6-Mc1re/e mice) with BLM. Wildtyp C57BL/6 mice were previously shown to be BLM-insensitive. Notably, only C57BL/6-Mc1re/e exhibited BLM-induced skin fibrosis as shown by quantitative and semiquantitative read-outs. These findings show that alpha-MSH via MC1 and catalase attenuates BLM-induced collagen synthesis in HDFs in vitro. Expression of functional MC1 in vivo protects against BLM-induced skin fibrosis.

P097

KdPT, an alpha-melanocyte-stimulating hormone-related tripeptide derivative, protects human melanocytes against oxidative stress-induced cell injury

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Oxidative stress is a key pathogenetic event in vitiligo. Substantial amounts of hydrogen peroxide are found in lesional skin of patients with vitiligo along with reduced catalase expression in situ. Moreover, melanocytes from patients with vitiligo are more susceptible to oxidative cell injury compared with melanocytes from healthy individuals. We wondered whether KdPT, a tripeptide derivative of alpha-melanocyte-stimulating hormone has protective effects against oxidative cell injury of melanocytes. This peptide has previously been shown to have antiinflammatory effects in vitro and in vivo. Using an vitro model of oxidant-induced cell injury, i. e. treatment with 4-tert-butylphenol (4-TBP), we show that KdPT dose-dependently reduces cell death and apoptosis of normal human epidermal melanocytes as measured by crystal violet assay, surface Annexin V immunostaining and cell death detection assay. Mechanistic studies revealed that KdPT per se increases expression of a panel of antioxidative factors and enzymes including the cytoprotective protein nuclear factor erythroid 2-related factor (Nrf2), heme oxygenase-1 but also of superoxide dismutase 1 (SOD1), SOD2 and catalase in a time-dependent fashion. Exposure of melanocytes to 4-TBP, as expected, also increased expression of Nrf2 and Nrf2-dependent enzymes as a result of an unsuccessful oxidative stress response of the cells. However, cotreatment with KdPT reduced the overall expression levels of these factors suggesting that the peptide confers antioxidative protection. In order to learn how KdPT may exert these beneficial effects we determined the expression of oligopeptide transporters of the SCL15 family in normal human melanocytes. Of note, PepT1 was previously shown to transport the KdPT-related tripeptide KPV into intestinal epithelia. Interestingly, PepT1 and PepT2 were undetectable while two other transporters, PHT1 and PHT2, were strongly expressed in human melanocytes. These preliminary data show that KdPT has indirect antioxidative properties and protects against 4-TBP-induced apoptosis of human melanocytes. Further studies are underway to extend these findings in a suitable in vivo or ex vivo models of vitiligo and to precisely define the putative role of PHT1 and PHT2 in this context.

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IGF-1 induces nuclear up-regulation of p-Akt and controls expression of nuclear transcription factor Forkhead box-O1 (FoxO1) levels in SZ95 sebocytes

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Introduction and objectives: A recent hypothesis in the pathogenesis of acne suggests that nutrition-related acne-inducing factors exert their action by reducing nuclear transcription factor Forkhead box-O1 (FoxO1) levels via activation of the phosphoinositide-3-kinase (PI3K) / Akt/ FoxO1 pathway. Previously we described an activation of this pathway with an early up-regulation of p-Akt (30 min) and delayed up-regulation of p-FoxO1 (90 min) after stimulation with 1M insulin-like growth factor-1 (IGF-1) in SZ95 sebocytes, associated with suppressed DNA synthesis and increased differentiation. The aim of this study was to further elucidate the mechanism of the yet hypothetical nutrigenomic regulation of insulinotropic western diet and thereby support the potential role of nuclear mobilization of FoxO1 in the pathogenesis of acne.

Materials and methods: SZ95 sebocytes were treated with 0.1M and 1M IGF-1 in a time-dependent manner and nuclear and cytoplasmic fractions were separated using the NE-PER Kit following manufacturer's instruction. Concerning nuclear FoxO activity measurement, sebocytes were transfected with FoxO reporter (a mixture of an inducible transcription factor responsive firefly luciferase reporter and constitutively expressing Renilla construct) and after overnight incubation and subsequent stimulation with 0.1M and 1M IGF-1 in the presence or absence of 50 M PI3-kinase inhibitor (LY294002), nuclear FoxO activity was determined by dual luciferase assay according to the manufacturer's instruction.

Results: We found a delayed nuclear up-regulation of p-Akt (90 min) after previously described early cytoplasmic up-regulation (30 min) upon stimulation with 1M IGF-1 by western blot, supporting the hypothesis of nuclear shift of p-Akt in the cytoplasm. In contrast to the cytoplasmic fractions revealing a significant up-regulation of p-FoxO1 after 90 min, the protein amounts of nuclear extracts were not sufficient to detect nuclear fractions of FoxO1 or p-FoxO1. Using the luciferase assay, nuclear FoxO activity was slightly decreased upon 0.1 M and 1M IGF-1 incubation after 60 and 90 min, while in the presence of the PI3-kinase inhibitor, nuclear FoxO levels significantly increased ($P < 0.05$).

Conclusion: Our data support the role of IGF-1 as an activator of p-FoxO1 via nuclear mobilization of FoxO1 after cytoplasmic activation of PI3K/Akt pathway resulting in translocation of p-Akt to the nucleus and thereby support the potential role of FoxO1 as a key molecule in the growth factor-related pathogenesis of acne.

P099

Prolactin potently modulates sebaceous gland function in vitro

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The production of sebum is the function most readily associated with the sebaceous gland, and hyperseborrhea has been implicated in the development of acne. Clinical evidence has long indicated that the hormone prolactin (PRL) may modulate sebaceous gland function, suggested by acne associated with hyperprolactinaemia. Despite this, the direct effect of PRL on sebaceous gland function in vitro has barely been studied. Given that the pilosebaceous unit expresses both PRL and PRL receptor (PRLR) we investigated the effects of PRL on sebaceous gland function using serum free full skin organ culture in the presence of PRL and the PRLR antagonist (δ1-9-G129R-hPRL). Hair bearing skin was obtained, from the frontotemporal region of the scalp of 3 female patients (aged 30–52 years) undergoing cosmetic surgical procedures. Investigation was restricted to female skin to avoid any confounding effect of sex. Sebaceous gland area, lipid production (oil red/sudan black histochemistry), sebocyte counts and 5α-reductase type 1 immunoreactivity were measured. Prolactin significantly increased sebaceous gland area and the percentage of sebocytes in the proliferating basal layer ($P < 0.05$). In contrast, PRL significantly decreased the percentage of mature, differentiated sebocytes ($P < 0.05$). Lipid production, including neutral lipids, triglycerides and cholesterol esters, was significantly increased by PRL ($P < 0.001$), as measured by oil red and sudan black staining intensity. These effects were abrogated by the addition of the PRLR antagonist. Similarly, PRL significantly increased sebaceous gland 5α-reductase type 1 immunoreactivity ($P < 0.001$), whilst co-administration with PRLR antagonist prevented these effects. In conclusion, PRL modulates sebaceous gland function in female skin in vitro, and the increased 5α-reductase type 1 IR suggests that PRL may stimulate the conversion of testosterone to 5α-dihydrotestosterone in the sebaceous gland. Given the reported sex-dependent effects of PRL on the hair follicle, these studies need to be repeated in male subjects. In addition, the effect(s) of PRL on peripheral androgen metabolism should be addressed in future studies, which could also elucidate whether PRLR antagonists may represent novel anti-acne agents.

P100

A role for the non-neuronal cholinergic system in cutaneous inflammation and stress

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Cholinergic signaling is increasingly accepted to play a key role in cutaneous homeostasis. Here we show that endogenous ligands to nicotinergic receptors are altered in atopic dermatitis and stress. We assessed the influence of the highly acute Trier social stress test (TSST) on neuro-immune plasticity (immunohistomorphometry) and the non-neuronal cholinergic system (NNCS) (quantitative RT-PCR) in skin biopsies by determining nerve fiber (NF)-density, NF-mast cell contacts, mast cell activation and expression of secreted mammal Ly-6/urokinase-type plasminogen activator receptor-related protein (SLURP) 1 and 2 (endogenous alpha-7-nicotinic acetylcholine receptor [α7nAChR] and α3nAChR ligands) and their receptors. Numbers of NGF+ dermal NF were increased in lesional AD compared to non-lesional skin. Numbers of PGP 9.5+ NF were increased in AD compared to control but numbers of Gap43+ growing NF were decreased. α7nAChR mRNA was significantly lower in lesional and SLURP-1 in non-lesional AD skin. TSST, mildly increased NGF + NF and PGP 9.5 + NF in non-lesional skin, but reduced NFs in lesional AD together with reduced numbers of degranulated mast cells. SLURP-1 and -2 mRNA levels decreased in control while α7nAChR and SLURP-2 levels increased in lesional AD. Thus, allergic inflammation and stress affect cutaneous neuro-immune interaction and NNCS marker expression with differential effects in healthy control, non-lesional AD and inflamed AD skin. To fine tune NNCS activation in inflammation appears a worthwhile target to control stress sensitivity.

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The non-neuronal cholinergic system (NNCS) and cutaneous inflammation in a mouse model for atopic dermatitis-like allergic inflammation

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The cholinergic system and the immune system are instrumental in a successful adaptation to physical and psychosocial stressors alike, but they also play a central part in the dysregulation of stress-vulnerability. Organs at the self-environment border such as the skin are particularly sensitive to disruption. In the work presented here, we compare the expression of elements of the non-neuronal cholinergic system (NNCS) in the skin of mice under inflammatory stress (experimental allergic dermatitis [AlD]) and under psychosocial stress (24 noise-related stress). Studies of mRNA expression patterns by microarray analysis showed a NNCS-regulation-associated cytokine-expression pattern of the skin in response to stress exposure. By immunhistomorphometry an attenuated expression of the acetylcholine (ACh) synthesizing enzyme ChAT was found in mast cells in AlD. Said expression corresponded to the level of the control group under additional stress exposure. At the same time the expression of the nicotinic ACh receptor α7nAChR was down-regulated in AlD while under stress a significant upregulation of the receptor was seen. The combination of stress and AlD showed an abolishment of the down-regulation of α7nAChR back at the control level. Likewise up-regulated in AlD was the expression of the pro-inflammatory cytokines tumor necrosis factor alpha (TNFα) and interleukin 1 beta (IL-1β), while the anti-inflammatory cytokines tumor growth factor beta (TGFβ) and IL-10 were down-regulated. Under stress the expression of TNFα, TGFβ and IL-10 did not show significant changes, but the expression of IL-1β was increased. 24 h noise stress exposure in combination with AlD revised this expression pattern. In summary, inflammation results in a dysregulation of the NNCS that is further disturbed by exposure to psychosocial stress and can affect the immune response. Further investigations will show the relevance of these results for the control of psychdermatologic disorders such as atopic eczema.

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Stress affects skin barrier function

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The skin, being the largest human organ, adopts the role of barrier between the environment and the body. This barrier is directed towards outside (physical penetration of foreign matter) as well as inward (loss of water and soluble substrates) challenges and also responds to psychosocial strain with a reduced function. Closure structures such as the epidermal tight junctions (TJ) that hold together the barrier-forming cells of the skin, the keratinocytes, may be key effectors of impaired barrier function. TJs comprise transmembrane closure proteins (eg claudins, occludins) and cytoplasmic plaque proteins (eg zonula occludens protein 1 [ZO-1]). We hypothesize that there is an influence of psychosocial stress on plasticity of TJ proteins. This would have particular relevance for the stress sensitivity of skin diseases with impaired barrier function, such as atopic dermatitis. We used an established mouse model of atopic dermatitis-like allergic dermatitis (AlD) in combination with noise-related stress to assess the barrier function by measuring the TEWL, inflammation, epidermal thickness, keratin-14 (K-14) expression and closure protein expression (occludin, ZO-1, claudin-1 [CLDN-1]) by immunofluorescence. First results show a dysfunction of the skin barrier in AlD, which increases under stress depending on neurotrophins. In the immunofluorescence a K-14+ hyperplasia of the epidermis is seen in AlD. As TJ-indicators of a disturbed barrier CLDN-1 seems sparsely available compared to the control group. Stress seems to enhance this dysregulation. Preliminary rtPCR data hint at a role of nicotinergic acetylcholine receptors. An inflammation and stress-associated down-regulation and redistribution of TJ proteins may therefore contribute to barrier disruption in AlD, especially under stress.

Dermatopathology

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Expression of the serine protease inhibitor of Kazal-type 9 in squamous cell carcinoma and actinic keratosis

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Recently, we discovered the serine protease inhibitor of Kazal-type (SPINK)9 as a Kallikrein-related peptidase (KLK)5-specific serine protease inhibitor expressed at palmoplantar sites of healthy individuals. As protease inhibitors and proteases are important factors in the pathogenesis of tumorgenesis and serve often as markers for different carcinomas we asked whether SPINK9 in expressed in squamous cell carcinoma and lesions of actinic keratosis.

Paraffin-embedded sections of cutanous squamous cell carcinoma and lesions of actinic keratosis were stained by specific anti-SPINK9 antibodies using standards methods.

SPINK9 immunoreactivity was detected at site of prominent hyperkeratosis of squamous cell carcinoma and lesions of actinic keratosis. Immunostaing was not detected in all investigated tissue sections.

Our results show that SPINK9 expression is not limited to palmoplantar sites as reported previously. When hyperkeratosis is present in squamous cell carcinoma and lesions of actinic keratosis SPINK9 expression might lead to hyperkeratosis by inhibiting KLK5. As SPINK9 was not present in all tissue sections of squamous cell carcinoma and lesions of actinic keratosis the benefit of SPINK9 antibodies as a diagnostic tool for detecting squamous cell carcinoma and lesions of actinic keratosis is not recommended.

P104

The role of Exportin-5 in miRNA processing in malignant melanoma

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MicroRNAs (miRNAs) are key players in the development of several kinds of diseases including cancer. It has already been shown that many miRNAs are deregulated in malignant melanoma, the most aggressive and fatal form of skin cancer, arising from melanocytes. In contrast to other cancers, the majority of miRNAs is upregulated in melanoma, leading to enhanced cell proliferation, migration and invasion. The reasons for this overexpression of miRNAs in melanoma are still not fully elucidated.

Therefore, we tried to find out whether changes in the miRNA processing machinery could be responsible for the enhanced miRNA expression. Recently, it was reported that the expression of the miRNA processing enzymes DICER1 and DROSHA is reduced during melanoma progression.
We investigated the potential role of the miRNA transporter Exportin-5 (XPO5) for the processing and maturation of miRNAs. In different melanoma cell lines, primary tumors and metastases of melanoma, XPO5 was found to be overexpressed compared to melanocytes. Thus, the effect of XPO5 downregulation and overexpression on different melanoma cell lines and melanocyte-like cell clones, respectively, was analysed.
Surprisingly, no major changes in the expression of a range of selected miRNAs could be detected, indicating that miRNA expression is not directly affected by modulation of XPO5 levels. Moreover, no substantial changes in neither cell proliferation nor migratory potential could be found after transfection. However, the capability of forming three-dimensional colonies out of one single adhesion-free cell was drastically influenced by XPO5 downregulation or overexpression, reflecting the connection between XPO5 and metastatic potential.
Taken together, these data suggest a role of the miRNA transporter XPO5 on metastasic potential in malignant melanoma.

P105

Melanoma inhibitory activity (MIA) plays a role in the induction of senescence in human melanocytes

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Malignant melanoma is a skin tumor which arises from the pigment-producing cells of the skin, the melanocytes. The protein melanoma inhibitory activity (MIA) has an influence on migration and invasion of melanoma cells, and thus plays a role in metastasis. We generated MIA deficient mice on the background of the Tg(Grm1)EPv transgenic melanoma mouse strain which is described to develop spontaneous melanomas with a short latency. Interestingly, EPv/MIA−/− mice showed an earlier onset of melanoma initiation compared to EPv/MIA wildtype animals. Compared to EPv/MIA wildtype mice, qRT-PCR analyses of nevi and melanoma from MIA deficient mice revealed a significant decrease of p21/CIP mRNA expression levels which is known as a cell cycle inhibitor and senescence marker. According to this, MIA also seems to be important in the early melanoma development in a protective way.
We recently described a MIA deficient melanoma cell line, HMB2-MIA. SA-ßGal positive cells could be found in the parental HMB2 cell line but not in MIA negative clones, further suggesting an impact of MIA on senescence induction.
In contrast to melanoma cells, normal human epidermal melanocytes (NHEM) secrete very low levels of MIA protein. We observed that cultured primary NHEM show increasing MIA levels according to higher cell culture passages correlating with an increase in senescent cells.
Treatment of NHEM cultured at low passages with recombinant MIA led to an increased expression of the cell cycle inhibitors p16/INK4A and p21/CIP, commonly known as senescence markers. Therefore, we assume that MIA is involved in the induction of senescence in melanocytes.
To understand the regulatory pathways, reporter gene assays were performed after addition of recombinant MIA. We observed decreased AP-1 activity in different melanoma cell lines after addition of recombinant MIA. The transcription factor AP-1 has been shown to be a regulator of the cell cycle by acting as a repressor of tumor suppressor genes, such as p53, p21/CIP and p16/INK4A.
These data suggest a potential role of MIA in the induction of senescence via MIA-dependent gene regulation.

P106

In vivo confocal laser scanning microscopy: diagnostic criteria for the differentiation of vesiculobullous skin disorders

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Background: In vivo confocal laser scanning microscopy (CLSM) is a modern non-invasive method for the investigation of dermal and epidermal lesions in high cellular resolution. Our aim was to define diagnostic CLSM-criteria for selected vesiculobullous skin disorders that may be helpful in daily clinical routine.
Methods: We examined patients with vesiculobullous skin disorders ($n = 32$) by using digital dermatoscopy (FotoFinder HD800 medicam) and in vivo CLSM (VivaScope 1500/3000). In a retrospective analysis of individual vesiculobullous skin disorders we were able to define several diagnostic CLSM-criteria specifically associated with the different disease entities. We report one representative case for each of the following diagnoses: i) bullous pemphigoid, ii) varicella zoster virus infection and iii) allergic contact dermatitis (positive patch-test reaction).
Results: Allergic contact dermatitis presented with intraepidermal microvesicles just below the stratum corneum, inflammatory infiltrate and spongiosis. The intact stratum corneum contained multiple nucleated keratinocytes as a sign of parakeratosis.
Varicella zoster infection was characterized by multichambered larger vesicles within the epidermis. Acantholytic cells and some lobulated-enlarged cells, with several nuclei and bright cytoplasm, corresponding to multinucleated giant cells, were surrounded by loose aggregates of keratinocytes, inflammatory cells and debris.
The bullous pemphigoid showed large bullae at the level of the dermoepidermal junction containing numerous inflammatory cells within the fluid. In the lower part of the stratum spinosum, an intercellular edema was interspersed with inflammatory cells. There were no abnormalities in the cellular structure or architecture of the upper stratum spinosum, stratum granulosum or stratum corneum.
Conclusions: Besides a good correlation between conventional histology and the results of confocal laser scanning microscopy, we could show distinguishing criteria for the above stated diseases, which may facilitate a non-invasive and immediate diagnosing, especially in vague or unusual clinical cases.

P107 (O27)

Cartilage oligomeric matrix protein contributes to the development of skin fibrosis

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Scleroderma is a heterogeneous autoimmune disease which is characterized by fibrotic alterations of the extracellular matrix (ECM) and subsequent hardening of the skin. Although the detailed etiology of the disease is still under investigation, it is agreed that the disease is triggered by trauma which results in vascular leakiness due to dysfunction of endothelial cell junctions. Immune cells are attracted to sites of damage, secreting soluble mediators (e.g. TGF-β), thereby creating a microenvironment that leads to attraction and sustained activation of fibroblasts and their transition to α-SMA expressing myofibroblasts. Myofibroblasts deposit huge amounts of ECM proteins and contribute to the development of a fibrotic ECM with an altered supramolecular arrangement of the collagen network and an aberrant composition of proteins.
We previously showed that the Cartilage Oligomeric Matrix Protein (COMP) is one of these proteins aberrantly regulated in scleroderma and deposited in large amounts into the dermal ECM of patients. COMP is known as an abundant protein in cartilage ECM involved in collagen fibril assembly, where one pentameric COMP molecule interacts with five collagen molecules. Reaction kinetics critically depend on the molar ratio between COMP and collagens.
To dissect the function of COMP in the development of fibrosis and to facilitate mechanistic studies, we used mouse models expressing different levels of COMP. In these, fibrosis of the skin was induced by consecutive intradermal injections of bleomycin, resulting in myofibroblast accumulation, excessive collagen production and rearrangement that led to dermal thickening.
In wildtype mice, this treatment induced high COMP production and deposition into the skin, resembling human scleroderma and thereby showing that the murine system is feasible for our purposes. By contrast, application of bleomycin to mice with global ablation of COMP (provided by A. Oldberg, Lund) resulted in an attenuated response, demonstrating the involvement of COMP in the fibrotic reaction.
To conduct mechanistic studies in a model resembling scleroderma, we generated a mouse model with a fibroblast-restricted forced expression of COMP. We have therefore cloned the murine COMP gene into the ROSA26 locus of C57Bl/6 mice genome and suppressed its transcription by placing an upstream STOP-cassette flanked by LoxP-sites. This strain was bred to mice expressing Cre-recombinase under the control of the proα2(I)collagen promoter and a far upstream enhancer that restricted Cre expression to fibroblasts. The stop cassette was removed by expression of Cre-recombinase in collagen I producing fibroblasts thereby allowing coincidental COMP transcription. These mice spontaneously developed certain characteristic features of skin fibrosis including dermal thickening and altered tensile properties. Ultrastructural analysis revealed alterations of the dermal collagen matrix reminiscent of those observed in wildtype skin after bleomycin treatment.
In accordance with these findings we hypothesize a model for the role of COMP during the development of fibrosis where activated myofibroblasts secrete increased amounts of collagen and COMP. In turn, increased COMP levels could interfere with the physiological collagen fibril assembly and thereby lead to the characteristic alterations of the collagen matrix as observed after induction of fibrosis either using bleomycin or by forcing expression of COMP in the dermis.

P108

Cartilage oligomeric matrix protein – an early determinant of skin elasticity

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The biological and mechanical properties of an extracellular matrix strongly depend on its composition as well as on the interaction of the proteins and other constituents involved. Cartilage Oligomeric Matrix Protein (COMP) is one of the non-collagenous proteins associated with collagen and until recently thought to be exclusively deposited in cartilage. We lately showed that COMP is also a component of healthy human skin. In the dermal extracellular matrix (ECM) COMP interacts with collagen I directly or indirectly via the fibril-associated collagens XII and XIV. To understand the biological function of COMP in the skin we compared early postnatal and adult skin of mice deficient for COMP with wildtype controls.
Abundant levels of COMP were deposited throughout the dermis of wildtype mice at 4–18 days after birth (P4-P18) but were not detectable after this time-frame. The function of this timely restricted appearance of COMP was investigated by taking advantage of mice with a global ablation of the protein (provided by A. Oldberg, Lund). Absence of COMP resulted in a significant thinning of the dermis at P7, a tilted orientation of the hair follicles and further in significant reduction of collagen XII levels, thereby indicating defects in early postnatal dermal morphogenesis. At the age of 6 weeks dermal thickness in COMP null mice had increased and did not differ from that of wildtype controls, however there were significant alterations regarding the mechanical properties and ultrastructure of the dermal collagen matrix. COMP null skin exhibited elevated elasticity compared to wildtype skin. Electron microscopy analysis of skin from COMP null mice revealed fibroblasts containing grossly dilated ER cisternae reflecting ER stress that is likely caused by protein retention. Collagen fibrils were abnormally tightly packed and of highly irregular shape. This abnormal fibril morphology and arrangement was persistent at least until the age of 6 month.
These results led us to conclude that COMP is a critical organizer in a tightly restricted time frame in early postnatal life, required for the correct assembly and supramolecular organization of the collagen network in the dermis. Absence of COMP results in structural alterations causing functional impairment that persists throughout adult life. We want to test the hypothesis whether COMP might fulfil this function by assisting the assembly of collagen I/XII 'fibrils' in the ER and facilitating their secretion.

P109

Role of CYLD in early melanoma development

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The tumor suppressor function of CYLD was first identified through genetic analysis of familiar cylindromatosis, which is an autosomal-dominant disorder characterized by a predisposition to benign skin tumors. Since then, mutation and depletion of CYLD have been associated with tumor development and progression of other cancers, such as breast cancer, renal cell carcinoma, colon cancer and malignant melanoma. Melanoma represents the most aggressive form of skin cancer with incidence rates increasing annually. In melanoma, expression of CYLD is down-regulated in consequence of increased activation of the transcription factor Snail1, resulting in increased proliferation and invasion of melanoma cells.
CYLD has a catalytic domain, which allows K-63 specific deubiquitination of proteins. A point-mutation in the cystein box (C601S) causes catalytical inactivation of the CYLD protein and affects its tumor suppressor function.
To study the effect of CYLD in melanoma tumorigenesis in vivo, CYLD knockout mice were crossed with Tg(Grm1)EPv mice that develop melanoma spontaneously. Analyses of this mouse model have shown that Cyld deficient mice evolve significantly earlier melanoma and have an accelerated tumor growth compared to the control group. Moreover, in vitro studies with human melanoma cell lines confirm that CYLD has a repressive effect on colony size.
To characterize through which mechanisms CYLD mediates its tumor suppressor function, we studied its effect on apoptosis, angiogenesis and lymphangiogenesis.
The cleavage of PARP (Poly (ADP-ribose) polymerase) was monitored by western blot analysis to test the influence of CYLD on apoptosis. Re-expression of CYLD in human melanoma cells increased the cleavage of PARP and therefore promotes apoptosis. In contrast, re-expression of the catalytical inactive CYLD C/S mutant did not lead to an increased apoptosis.
The metastatic spread of malignant melanomas occurs primarily through lymph nodes. Using tube formation assays and qRT-PCR analyses, the role of CYLD in angiogenesis as well as lymphangiogenesis was examined. Both the control and CYLD expressing melanoma cells were able to form tubes. Moreover, no difference in the expression of angiogenesis and lymphangiogenesis markers could be observed on mRNA level. These results assume that CYLD has no impact on angiogenesis and lymphangiogenesis in vitro.
Altogether, these findings could contribute to a better understanding of the tumor suppressor function of CYLD in malignant melanoma.

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P110 (O12)

Intra-individual genome expression analysis reveals a specific molecular signature of psoriasis and eczema

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Previous attempts to gain insight into the pathogenesis of psoriasis and eczema by comparing their molecular signatures were hampered by the high inter-individual variability of these complex diseases. Using a cohort of patients affected by both psoriasis and different clinical variants of eczema simultaneously ($n = 20$), we confirmed that unsupervised clustering of whole genome expression resulted in patient- rather than disease-related grouping. However, intra-individual comparison of the molecular signatures of psoriasis and eczema identified genes as well as signaling pathways regulated in common and unique for each disease across all patients. Genes regulated exclusively in psoriasis belonged to the epidermal compartment (differentiation and antimicrobial response), glucose and lipid metabolism such as iNOS, as well as to the immune system, including Th17 responses, IL-10 family cytokines IL-19 and IL-20, and IL-36A/G. Unique genes in eczema related to an impaired epidermal barrier, reduced innate immunity, increased IL-6 and a Th2 signature. Based upon this comprehensive picture of the pathogenesis of psoriasis and eczema, a disease classifier consisting of 15 genes was created. In an independent cohort of eczema ($n = 10$) and psoriasis ($n = 10$) patients, respectively, this classifier diagnosed all patients correct and identified one initially mis-diagnosed patient.

P111

IL-36γ is a highly specific marker for psoriasis skin lesions

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Background: The IL-36s are a newly identified cytokine family associated with the IL-1 cytokines, previously designated as IL-1Fs. Each cytokine binds specifically to the IL-36 receptor (IL-36R, also named IL-1Rrp2 or IL-1RL2), which leads to the recruitment of IL-1RAcP and activation of NF-kB. The IL-36s have been shown to induce many cytokines which play a central role in psoriasis including TNFα, IL-12, IL-17, and IL-23. Keratinocytes produce IL-36s upon stimulation with IL-17 and polyIC.
Objective: To evaluate the value of IL36γ as a potential marker for psoriasis.
Patients and methods: In the first step, gene expression analyses of lesional skin biopsies taken from patients with active skin diseases (Psoriasis/Pso, atopic dermatitis/AD), lichen planus/LP) and healthy controls/HC ($n \geq 30$, respectively) were performed. Results were confirmed by immunohistochemistry on the protein level.
Results: Statistical analyses of gene expression analyses revealed a set of markers which are specifically expressed in psoriasis, but not in other inflammatory skin diseases. Among these, IIL36γ was the most specific marker. These results were confirmed by immunohistochemistry, where IL36γ was strongly expressed in the upper epidermal layer of psoriasis specimens, but only weakly in the control samples (AD, LP and HC).
Discussion: IL36γ is assumed to play a central functional role at the immunological interface between innate and adaptive immunity. Our results demonstrate that IL36γ specifically is expressed in psoriasis skin lesions, but not in the other inflammatory diseases analyzed. We conclude that IL36γ obviously is involved in the proinflammatory network of psoriasis and might provide a potential biomarker for this disease.

P112

Immunohistochemical studies on hornerin in inflammatory skin diseases

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Hornerin is a 245 kDa S100 fused-type protein, which shares features with filaggrin. It is part of the human stratum corneum and undergoes complex processing during terminal differentiation. Its role concerning inflammatory skin diseases such as atopic dermatitis and psoriasis is not fully understood. We analysed hornerin expression in skin biopsies of patients with atopic dermatitis comparing to healthy controls, lesions of psoriasis and allergic contact dermatitis. Affinity-purified anti-HRNR antibodies raised against different domains of the HRNR molecule were used and the tissue was analysed via immunohistochemical staining.
We found that human HRNR was reduced in inflammatory skin diseases compared to healthy controls. Surprisingly higher expression levels were observed in skin biopsies of patients with atopic dermatitis compared to psoriasis and allergic contact dermatitis suggesting a putative role of hornerin in psoriasis and allergic contact dermatitis. Further studies are necessary to understand the role of hornerin in inflammatory skin diseases and its function in healthy skin.

P113

Mast cells enhance the antimicrobial potential of keratinocytes in response to *Pseudomonas aeruginosa*

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The skin immune system contains both, innate and acquired immune responses to defend the organism against microbes or microbial substances. Keratinocytes (KCs) form the outermost layer of the skin and function not only as a mechanical barrier to invading pathogens but also participate in the innate immune defense by producing antimicrobial peptides (AMPs) that inactivate a wide variety of microorganisms. Mast cells (MCs) are situated within the skin compartment and are described to have a regulatory role on skin homeostasis as well as on the immune function of the skin. By releasing various mediators, derived from their granules, MCs are able to influence the immune response by recruiting and/or activating other immune cells. MCs have also been reported to produce and release AMPs by their selves and therefore support the role of KCs. Whether and how MCs and KCs interact to orchestrate the AMP machinery is not well known. Therefore, the aim of the present study was to explore the relationship between MCs and KCs under pathophysiological conditions. To investigate the influence of MCs on KCs we established a KC-MC co-culture system that was challenged with *Pseudomonas aeruginosa* (PA) as a typical and clinical relevant skin pathogen. In this co-culture system we could show that upon bacterial challenge KCs alone elicited only a minor antibacterial effect. More than 90% of the bacteria still survived after 3 h of co-culture compared to control bacteria. Most interesting, when KCs were cultured together with MCs the antibacterial effect was boosted upon an effect that killed up to 50% of the bacteria. Using a qRT-PCR approach we could identify an upregulation of AMPs derived from KCs stimulated by the co-culture with MCs. To analyse the molecular mechanisms responsible for these antibacterial promoting effects we did multiplex ELISA studies that revealed and increased IL-6 production from MCs in the co-culture system. To prove that increased IL-6 levels lead to enhanced antimicrobial response of KCs IL-6-deficient MCs were used in the co-culture experiments. Most notably, IL-6-deficient MCs failed to enhance the antibacterial capacity of KCs which was comparable to KCs culture without MCs. To conclude, we could show that MCs have a regulatory role in the expression of AMPs by KCs to improve innate skin immunity against bacteria.

P114

IL-19 is a novel component of the pathogenetic IL-23/IL-17 cascade in psoriasis

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Cytokines play a key role in the pathogenesis of psoriasis, a common chronic inflammatory disease with characteristic skin alterations functioning as a model of immune-mediated disorders. Importantly, out of 30 individually quantified cytokines we revealed the strongest differential expression between psoriatic and healthy skin for IL-19. Cutaneous IL-19 overproduction was reflected by elevated IL-19 blood levels that correlated with psoriasis severity. Accordingly, anti-psoriatic therapies substantially reduced both cutaneous and systemic IL-19 levels. IL-19 production was induced in keratinocytes by IL-17A and further amplified by TNF-α, IL-22, and IL-19 itself, the latter demonstrating a positive feedback loop. Among skin cells, keratinocytes were found to be important targets of IL-19. IL-19 alone however regulated only a few keratinocyte functions. While increasing the production of S100A7/8/9 and, to a moderate extent, also IL-1β, IL-20, CXCL8, and MMP1, IL-19 had no clear influence on the differentiation, proliferation or migration of these cells, Instead, IL-19 amplified many IL-17A effects on keratinocytes, including the induction of β-defensins, IL-19, IL-23, and Th17-cell- and neutrophil-attracting chemokines. In summary, IL-19 as a novel component of the IL-23/IL-17 axis strengthens the IL-17A action and, simultaneously, might be a biomarker for the activity of this axis in chronic inflammatory disorders.

P115 (O19)

Reasons for the different frequencies of cutaneous viral infections in atopic dermatitis and psoriasis

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Atopic dermatitis (AD) and psoriasis are two of the most common chronic diseases. Although both patient groups show strong skin inflammation and barrier disruption, only AD patients frequently suffer from cutaneous viral infections. The mechanisms underlying the distinct susceptibilities to these pathogenetic and often life-threatening infections are still unknown. Now, we found that the expression of numerous antiviral proteins (AVPs) was much higher in psoriatic than in AD lesions and healthy skin and dominantly present throughout the epidermis. Out of 27 individually quantified cytokines in psoriatic lesions, IL-29 was the only mediator whose expression correlated with the AVP levels. Notably, IL-29 was absent in AD and healthy skin. The relationship between IL-29 and AVP presence in psoriatic lesions was direct because (i) this cytokine increased AVP production in primary human keratinocytes and three-dimensional human epidermis models, (ii) IL-29 injection into mouse skin enhanced its cutaneous antiviral competence, and (iii) the neutralization of IL-29 in psoriatic lesions reduced their AVP expression. Importantly, IL-29-induced AVP expression levels correlated with the inhibition of virus infection of IL-29-treated keratinocytes. Regarding the cellular sources of IL-29, we demonstrated for the first time that Th17-cells and, to a much smaller extent, Th1-cells produced high amounts of IL-29, and we characterized the features of IL-29 production and regulation in these cells. Accordingly, the supernatant of Th17-cells IL-29-dependently increased the AVP expression and inhibited virus infection of treated keratinocytes. In summary, our data strongly suggest that Th17-cell derived IL-29 mediates the robust antiviral state upon psoriatic skin. Both, the limited number of Th17-cells as well as the cutaneous presence of IL-4, an inhibitor of IL-29 production, are responsible for the high incidence of cutaneous viral infection in AD patients. By demonstrating an antiviral function of Th17-cells our work additionally reveals a new aspect of the immune system.

Epidemiology

P116

A pilot study on DNA methylation and RNA expression in diverse tissues reveals epigenetic changes in atopic dermatitis

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Epigenetic alterations are increasingly recognized as mechanisms for disease-associated changes in genome function and important risk factors for complex diseases. In order to identify disease-associated methylation differences for atopic dermatitis (AD), we investigated DNA from lesional and non-lesional epidermis from 17 AD patients and 28 healthy controls using the 27K Human Methylation Bead Chip. To elicit functional links we examined epidermal mRNA expression profiles with the HT-12v3 Expression Bead Chip. For a subset of study subjects, methylation and expression analysis was additionally performed in whole blood, T-cells and B-cells. All results were validated with the EpiTyper MassARRAY and RT qPCR, respectively. Our analysis across tissues showed that intra-individual inter-tissue variation in DNA methylation exceeds inter-individual variation in any given single tissue. In particular, epidermis and blood cells appear to have very distinct methylomic profiles. Striking differences in methylation were observed between lesional epidermis from patients and healthy control epidermis for various CpG sites, most of which were located in genes relevant for epidermal differentiation and immune response. These methylation alterations were discordant in skin and blood samples, suggesting that blood cannot serve as a surrogate for skin tissue. Using an integrative approach, significant correlations between CpG methylation degrees and mRNA expression levels of genes in-cis were observed. This pilot study provides a starting point for future investigations of epigenetic mechanisms in AD.

P117

Chronic pruritus in hemodialysis patients in Germany: prevalence and risk factors

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Worldwide, estimated two million persons suffering from end stage renal disease (ESRD) are depending on hemodialysis. These patients frequently develop chronic and often therapy-refractory pruritus. Epidemiological data on the prevalence of this type of pruritus are sparse. The pathogenesis also still remains unknown. Decades ago, up to 85% of hemodialysis patients were affected by pruritus, but due to improved hemodialysis techniques its prevalence appears to have decreased. New research has shown that the prevalence of chronic pruritus in hemodialysis patients greatly varies between country and dialysis center. However, representative epidemiological studies do not exist yet. We performed a randomized, representative cross-sectional study in Germany. Calculation of power and sample-size were conducted. Reliability of selected study instruments was tested within 20 hemodialysis patients in a pre-study. A cluster-sample of 25 dialysis centers was randomly selected according to geographical regions in Germany (North-West, North-East, South-West and South-East). The primary study outcome was the prevalence of pruritus. Secondary outcome measures were characteristics of pruritus, laboratory findings, dialysis techniques, comorbidities and health-related Quality of Life (HRQOL). In a first step socio-demographic data, existence of pruritus, general health status (SF-12 questionnaire), personal hygiene, anxiety and depression (HADS questionnaire), sleeping disorders and other physical complaints were assessed in all patients of the selected dialysis centers. Comorbidities (Charlson Comorbidity Index), aetiology of ESRD, characteristics of hemodialysis and techniques as well as laboratory and dialysis parameters of all patients were also included. Those who reported chronic pruritus completed a pruritus specific questionnaire including data on chronic pruritus such as frequency, course, duration, severity, localization, characteristics, previous therapy and pruritus-related Quality of Life (ItchyQoL questionnaire). All patients affected by pruritus were examined by a dermatologist in order to gain information on the patients skin status and possible present skin diseases which might be associated with pruritus. Data collection in all dialysis centers was finished successfully in September 2013. 850 hemodialysis patients were included in the study. We present results of the prevalence study including e.g. characteristics of pruritus, associated comorbidities and cofactors as well as HRQOL.

P118

PCR-ELISA-based identification of dermatophytes isolated from the scalp of children with tinea capitis attending Mbarara Regional Referral Hospital in Uganda

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Objective: Tinea capitis is a dermatophyte infection which is common among pre pubertal children in sub-Saharan Africa and mainly caused by Trichophyton (T.) and Microsporum (M.) species. Accurate identification is challenging as conventional methods like culture and microscopy are slow and mostly based on morphological characteristics which make them less sensitive and specific. Modern methods, like PCR-ELISA assay, are gaining acceptance and are quick as well as accurate. The aim of this study was to investigate the clinical patterns of tinea capitis and to accurately identify the most common causative dermatophytes affecting the scalps of children aged 1–16 years attending Mbarara Regional Referral Hospital (MRRH), Mbarara, East Africa, using both conventional and PCR-ELISA methods.
Method: 115 clinical samples from children from western Uganda attending Mbarara University Skin clinic with symptoms suggestive of tinea capitis were analysed in two mycology laboratories in Germany (University of Jena, and Mölbis). Dermatophtyes were identified using conventional laboratory methods (direct microscopy, culture) and PCR-ELISA assay.
Results: *T. violaceum* was identified as the most common causative agent with an incidence of 56.52% (65/115), followed by M. audouinii with 13.04% (15/115), T. soudanense with 2.61% (3/115), and T. rubrum with 2.61% (3/115). Direct microscopy with Blancophor staining exhibited increased sensitivity up to 82.61%, (95/115) and 84.3% (97/115) compared to culture and PCR-ELISA, respectively. The sensitivity and specificity of the PCR-ELISA for detecting dermatophytes when compared with culture as gold standard was 90.72% and 96.7% with positive and negative predictive values of 83.3% and 62%, respectively (Pearson $\chi^2(1) = 0.4194$). Moreover, it was found that the fungi mainly caused an endothrix invasion of the afflicted hairs.
Conclusion: Tinea capitis is of public health significance and commonly caused by anthropophilic fungi. In this study, PCR-ELISA as a molecular biological method for direct identification of dermatophyte DNA in clinical samples, proved to be a highly sensitive, specific, rapid and reliable tool that is independent of time consuming culture evaluation (takes up to 4 weeks) and biochemical methods. *T. violaceum* was identified as the most common causative agent with an incidence of 56.52%. The ability to identify dermatophytes up to species level is a step forward in solving the problems of taxonomic ambiguity associated with dermatophytes and importantly ensuring that correct therapies are initiated early for these patients.

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A prospective epidemiological study of infantile hemangioma in an Austrian tertiary referral center ('the transdanubian cohort')

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Introduction and objectives: Epidemiologic data on incidence, course, risk factors and response to treatment of infantile hemangiomas (IHs) are mainly derived from retrospective studies of small populations. In this prospective study with 1755 newborns in our tertiary referral hospital we evaluated prevalence, incidence, risk factors and treatment outcome.
Methods: With parents' informed consent 1755 children born between July 2011 to June 2012 were included. Children were followed within the first year of life for occurrence of IHs. Dependant on size, speed of progression and localization of IHs, management strategies were either observation with repeated clinical controls, treatment with Dye-laser, systemic propranolol, topical timolol or cryotherapy.
Results: The observation period of our study ended by June 2013; statistical analyses are currently still in progress and more results will follow.
Of 2036 newborns we were able to include 1755 (86%) in our database: 48% females, 52% males. We found 154 IHs or precursor-lesions in 113 children which marks a prevalence of 6.4%, with a tendency towards females (54% females, 46% males).
IHs were located most frequently on the trunk (74 IH, 48.1%), followed by extremities (52 IH, 33.8%), head and neck (28 IHs, 18.2%). In 12.3% IHs were located in risk areas such as fingers, toes, nose, lips or genital area. Most of the children had one IH (77%), 18 children 2 IHs (16%) and 8 children had three or more IHs (7%). Prevalence was higher in multiple pregnancies (12.5%) versus single pregnancies (6.1%), and, furthermore, appeared inversely correlated with time of birth and children's weight in order to the numbers of IHs per child.
100 IHs (64.9%) were followed by clinical observation without any therapeutic interventions. 41 IHs (26.6%) received monotherapy, and 13 IHs (8.4%) combined therapy because of insufficient response or progression of growth. The most frequently used therapeutic option was DYE-laser (34 IH), followed by cryotherapy (18 IH), systemic propranolol (7 IH) and topical timolol (7 IH).
Conclusions: In our well controlled cohort of 1755 infants, followed from birth through the first year of life, we found a prevalence of IHs of 6.5%. Risk factors appeared to be multiple pregnancies, premature birth, low birth weight and female sex. Furthermore, in our cohort more than 60% of IHs could be managed by observation only with active non-intervention, while less than 40% received specific treatments, individually tailored to patientsneeds.

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A genome-wide association study of atopic dermatitis identifies loci with overlapping effects on asthma and psoriasis

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Atopic dermatitis (AD) is a strongly heritable disease, which frequently occurs in childhood and is often accompanied by asthma and/or allergic rhinitis (AR). Linkage and association studies have indicated shared genetic susceptibility regions of AD and other chronic inflammatory diseases. We present here a genome-wide association study (GWAS) of childhood-onset AD in 1563 European cases with known asthma status and 4054 European controls. Signals from the initial scan was replicated in a second panel of 2286 cases and 3160 controls of European descent. We identified four loci consistently associated: the epidermal differentiation complex (EDC), the genomic region proximal to LRRC32, the RAD50/IL13 locus and the major histocompatibility complex (MHC), reflecting action of the classical HLA alleles. We observed variation in the contribution towards co-morbid asthma for these regions of association. We further observed considerable overlap between AD and psoriasis together with variable coincidence between AR and asthma. Our results indicate that the pathogenesis of AD comprises both epidermal barrier and immune response defects with both specific and overlapping effects at individual loci.

P121

Genome-wide comparative analysis of atopic eczema and psoriasis gives insight into disease mechanisms

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Atopic eczema and psoriasis are common chronic inflammatory skin diseases with strong heritability. Genome-wide association studies in these diseases have identified shared genetic risk loci including the epidermal differentiation complex and the MHC region. However, atopic eczema and psoriasis rarely co-occur within the same patient, indicating mutually exclusive pathogenic features. Shared and exclusive genetic risk loci within eczema and psoriasis may represent overlapping pathophysiological mechanisms and important switch points in pathways determining susceptibility to one or both diseases. Using imputed genome-wide association data from cohorts including over 18 000 individuals, we aimed to systematically compare and contrast eczema and psoriasis on a genomic level using methodology developed from meta-analysis techniques. This approach has identified antagonistic loci within the EDC on 1q21.3 and the cytokine cluster on 5q31.1 and provided new insight into pathogenic mechanisms within inflammatory skin disease.

P122

Patients' preferences for the treatment of basal cell carcinomas: cure and cosmetics count

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Background: Basal cell carcinomas (BCC) are the most common skin tumours of Caucasians worldwide with increasing incidence. Treatment options include simple excision, micrographic surgery, topical treatment with imiquimod or 5-fluorouracil, photodynamic therapy, radiotherapy and vismodegib for unresectable and metastasizing BCC.
Objectives: Our aim was to investigate patients' preferences for treatment of BCC, using discrete choice experiments.
Methods: Participants with BCC attending outpatient dermatology clinics at the University Medical Center Mannheim completed a survey containing sociodemographic, socioeconomic and disease-related information and conjoint analysis exercises. For generation of the discrete choice scenarios, all available treatment options for BCC except vismodegib were decomposed into outcome (cure rate, recurrence rate, cosmetic results, risk of temporary complications, risk of permanent complications) and process attributes (kind of treatment, treatment location, anesthesia, way of wound closure, duration of wound healing, individual costs) and attribute levels. Hypothetical treatment scenarios were generated with Sawtooth software, and individuals were repeatedly asked to choose their preferred option among pairs of options. Relative importance scores (RIS) were calculated for each attribute and averaged across the sample. Subgroup analyses were performed according to sociodemographic, socioeconomic and disease-related characteristics.
Results: 124 participants were recruited (56.5% males, mean age 69.1 years). 14.5% suffered from recurred BCC, and 42.7% reported at least one previous BCC. 87.1% of the tumours were located on the head or neck. The treatment attribute considered most important in the whole study sample was recurrence rate (RIS = 17.28), followed by cosmetic result (RIS = 16.90) and cure rate (RIS = 15.02). No significant differences were noted with respect to age, gender and income. Participants with BCC

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on the head or neck were more interested in cosmetic outcome than others. Participants who were highly concerned about their tumour as well as participants who classified their general state of health as poor worried more about the risk of temporary complications.
Conclusion: Recurrence and cure rate as well as the cosmetic result are the most important attributes for patients suffering from BCC treated in a University Hospital setting, regardless of gender, age and income. The treatment process and location appear to be comparably less important.

Genetics

P123

A novel mouse model reveals the pivotal role of SNAP29 in epidermal differentiation

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In differentiating keratinocytes the spatially and temporally controlled secretion of lipids, proteases, and protease inhibitors by lamellar bodies is an essential process for the formation of the epidermal barrier and a well-regulated desquamation of the skin. SNAP29 is a SNARE protein presumably involved in the maturation of lamellar bodies. Loss-of-function mutations in the Snap29 gene cause CEDNIK (cerebral dysgenesis, neuropathy, ichthyosis, keratoderma) syndrome, a rare human genodermatosis, associated with disturbances in lamellar body maturation. In this study, we created a Snap29 deficient mouse model for CEDNIK syndrome to investigate the role of SNAP29 in epidermal differentiation. Snap29 deficient mice exhibit neonatal lethality. Their skin showed morphological abnormalities in the epidermal structure like ichthyosis with para-hyperkeratosis and acanthosis accompanied by a disturbed formation and/or maturation of lamellar bodies as well as a reduced number of hair follicles. The causative loss of Snap29 expression is accompanied by enhanced expression of keratin 14 (expanded to suprabasal layers), and keratin 6 (indicating hyperproliferation) and by reduced expression of involucrin (cornified envelope structural protein), TGN46 (a marker of lamellar bodies and trans-Golgi network), and kallikrein 7 (lamellar body cargo) as assessed by immunohistochemistry. Our results provide deeper insight into the essential role of SNAP29 in the development of a functional epidermis, its contribution to intracellular transport processes during keratinocyte differentiation and its necessity for postnatal survival.

P124

An unusual mutation in the XPG gene leads to an internal in-frame deletion and a XP/CS complex phenotype

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Seven xeroderma pigmentosum (XP) complementation groups (XP-A to XP-G) and a variant form with a defect in translesion synthesis have been identified. Patients belonging to XP complementation groups B, D, and G can exhibit XP symptoms combined with Cockayne syndrome (CS) symptoms (XP/CS complex phenotype) indicating a role of the respective protein in nucleotide excision repair (NER) as well as in basal transcription. So far, only 19 patients with a defect in the XPG gene and 25 different disease-causing mutations have been described worldwide. Here, we report a 5-years-old Turkish girl with a severe XP/CS complex phenotype. Primary patient skin fibroblasts revealed a severely reduced post UV-cell survival as assessed by MTT-assay. Wildtype fibroblasts showed a relative NER capacity of 16% while the patient cells exhibited a decreased repair capacity of only 0.1% as determined by host cell reactivation (HCR). This repair defect was corroborated by markedly reduced unscheduled DNA synthesis (UDS) of 3%. Cotransfection of a XPG cDNA containing plasmid assigned the patient to complementation group G. We identified an unusual homozygous genomic complex XPG deletion of 6.7 kbp. XPG cDNA showed two resulting variants with skipping of exons 2–5. Variant one consisted of an in-frame deletion of the last four bases of exon 1 and the entire exons 2–5. The predicted protein harbors aa1–28 continuing with aa176–1186 resulting in a truncated XPG protein missing aa29–175. In variant two the last 40 bp of exon 1 were deleted together with exons 2–5 in-frame leading to a truncated XPG protein lacking aa17–175. Western blot analyses showed a clearly detectable but reduced expression of a truncated XPG protein compared to wildtype fibroblasts. For further analysis, the mutated protein variant one – due to the shorter in-frame deletion – was cloned into the pcDNA3.1(+) expression vector as well as into the pcDNA3.1(+) expression vector containing a C-terminal eGFP-tag. Overexpression of this protein variant in HeLa cells further confirmed that the mutated protein is stable as its expression increased over time (24–72 h). We demonstrated the nuclear localization of this mutated XPG protein variant by overexpression of the eGFP-tagged variant in HeLa cells applying fluorescence microscopy. Complementation ability of this variant in XPG-deficient XP20BE was assessed by HCR. Cotransfection with wildtype XPG cDNA resulted in restored NER, while cotransfection with the patient's variant one cDNA led to a repair capacity <0.1%. This indicates that the repair function of variant one is totally abolished. The CS symptoms of our patient suggest a dual loss of protein function with additional impairment of XPG's structural role during basal transcription. This is supported by the fact that the mutated protein variants lack aa29–175 or aa16–175 which are large parts of the N-terminal TFIIH interaction domain. With our findings, we can now narrow down the N-terminal essential TFIIH interaction domain of XPG from aa1–377 as previously reported to aa29–175.

P125 (O30)

A common atopy-associated variant in the Th2 cytokine locus control region impacts transcriptional regulation and alters SMAD3 and SP1 binding

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Background: Type 2 immune responses play major pathogenic roles in atopic diseases. They are directed by Th2 cells and characterized by the signature cytokines IL4, IL5 and IL13. Single nucleotide polymorphisms (SNPs) in the human Th2 cytokine locus and in particular variants in an intronic region of the DNA-repair gene RAD50, including the RAD50 DNase1 hypersensitive site 7 (RHS7), have been robustly associated with atopic traits in genome-wide association studies (GWAS). Other functional variants in the IL13 gene have been intensely studied. However the functional causative variants for the IL13 independent RAD50 signal have not been identified yet.
Objective: This study aimed to characterize the functional impact of the common atopy associated polymorphism rs2240032 located in the human RHS7 on cis-regulatory activity and on differentially binding transcription factors.
Methods: Differential transcription factor binding was analyzed by electrophoretic mobility shift assays (EMSAs) with Jurkat T-cell nuclear extracts. Identification of differentially binding factors was performed using mass spectrometry (LC-MS/MS). Reporter vector constructs carrying either the major or minor allele of rs2240032 were tested for regulating transcription activity in Jurkat and HeLa cells.
Results: Allele-specific binding of SMAD3, SP1 and additional putative protein complex partners was identified at rs2240032. The atopy risk allele exhibited decreased SMAD3 and SP1 binding and showed significantly enhanced promoter activity compared to the non-risk allele in Jurkat cells. We further demonstrate that rs2240032 is located in an RHS7 subunit which itself encompasses repressor activity and might be important for the fine-tuning of transcription regulation within this region.
Conclusion: The human RHS7 critically contributes to the regulation of gene transcription, and the common atopy-associated polymorphism rs2240032 impacts transcriptional activity and transcription factor binding.

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3exon replacement for COL17A1

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Epidermolysis bullosa (EB) is an inherited skin disorder in which minor trauma leads to blistering of skin and mucous membrane. Depending on the level of tissue cleavage EB has been divided in four main groups: EB simplex, dystrophic EB, junctional EB and kindler syndrome. Mutations in the COL17A1 gene, coding for type XVII collagen, lead to the junctional form of EB (JEB), with cleavage within the lamina lucida. There are different strategies for EB gene therapy under investigation.
Currently, a major focus lies on full-length cDNA therapy using retroviral delivery systems. However, because of the large size of the COL17A1 mRNA (~5.6 kb), we decided to use the Spliceosome Mediated RNA Trans-splicing (SMaRT) technology with the main advantage, that only parts of the gene of interest are replaced, thereby circumventing viral packing limitations.
To repair mutations at the mRNA level, SMaRT uses the cellular splicing machinery to replace one or more exons. In detail, for the correction of COL17A1 we engineered a RTM (RNA trans-splicing molecule) including (a) the wild-type coding region of the gene protion to be replaced, in our case Exons 34–56 of the COL17A1 gene, (b) essential functional splice sites and (c) a target recognition sequence/ binding domain, which hybridizes to the endogenous COL17A1 pre-mRNA. As the binding domain is crucial for trans-splicing specificity and efficiency, a fluorescence based RTM screen on random binding domains was performed. Co-transfection of a binding domain library with a target molecule resulted in the identification of highly functional binding domains (83.4% efficiency in RTM15 and 80.6% efficiency in RTM8).
On endogenous level, two RTMs, including exons 34–56 of the COL17A1 gene and the previously identified, highly functional binding domains, were integrated into immortalized GABEB (generalized benign epidermolysis bullosa) keratinocytes, which represent the junctional form of EB. For both constructs, successful trans-splicing was detected on mRNA level and also in immunofluorescence microscopy a positive staining for COL17A1 was observed.
In consideration of our findings we conclude, that SMaRT is a potential tool for the correction of COL17A1 mutations.

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Natural gene therapy in dystrophic epidermolysis bullosa

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Revertant mosaicism has been reported in several inherited diseases, including isolated cases of epidermolysis bullosa (EB). DEB is a particular subtype of EB associated with mutations in the collagen VII-gene (COL7A1). We describe the largest cohort of seven patients with revertant mosaicism and dystrophic EB (DEB) and determine the underlying molecular mechanisms. We show for the first time that revertant mosaicism occurs both in dominantly and recessively inherited DEB. We found that both null mutations resulting in complete loss of collagen VII and severe disease, as well as missense or splice-site mutations associated with a milder phenotype, were corrected by revertant mosaicism. The mutation, subtype and severity of the disease are thus not decisive for the presence of revertant mosaicism. Although collagen VII is synthesized and secreted by both keratinocytes and fibroblasts, evidence for reversion was only found in keratinocytes. The reversion mechanisms included back mutations/mitotic recombinations in 70% of the cases and second-site mutations affecting splicing in 30%. Our data imply that in patients heterozygous for COL7A1 mutations, the reversion preferably occurs through correction of the mutation itself through back mutations or mitotic recombination, whereas in homozygous patients second-site mutations occur. We conclude that revertant mosaicism is more common than previously assumed in patients with DEB, and our findings will have implications for future therapeutic strategies using the patient's naturally corrected cells as a source for cell-based therapies.

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The top skin-associated genes: a comparative analysis of human and mouse skin transcriptomes reveals significant differences in signaling and immunity

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The mouse represents a key model system for the study of the physiology and biochemistry of skin. Comparison of skin between mouse and human is critical for interpretation and application of data from mouse experiments to human disease. Recently, we used a genome-wide database of gene expression representing 105 different adult human tissues to identified genes highly expressed in skin, with no, or limited expression elsewhere – human skin-associated genes (hSAGs). Analysis of our set of hSAGs allowed us to generate a comprehensive molecular characterization of healthy human skin. Here, we used a similar database to generate a list of mouse skin-associated genes (mSAGs). A comparative analysis between the top human ($n = 687$) and mouse ($n = 876$) SAGs revealed a total

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of only 27 percent (188 genes of the 687 possible pairs) identity between the two lists. The majority of shared genes encode proteins that participate in structural and barrier functions, including members of the keratin protein family (KRT), cell-cell junction proteins (CLDN1, DSG1, DSP, DST, KLK5), and components of the cornified envelope (FLG2, LOR). Analysis of the top functional annotation terms revealed overlap for morphogenesis, cell adhesion, structural and signal transduction terms but significant enrichment of wound healing and response to steroids in human skin, and to muscle and carbohydrate binding in mouse skin. Genes identified as expressed exclusively in human skin included the antimicrobial peptide dermcidin (DCD), two secretoglobins (SCGB2A2 and SCGB1D2) and the interleukin 37 (IL1F7 / IL37), whereas the exclusive mSAGs list contained 5 genes from the selection and upkeep of intraepithelial T cells (Skint) gene family (Skint 3, 4, 9, 10, 11) and three genes from the interferon activated gene family (Ifi202b, Ifi204 and Ifi205). Our study represents one of the most comprehensive molecular comparisons of human and mouse skin to date, illustrating the diversity between the molecular make up of skin of both species and granting a probable explanation, why results generated in murine in vivo models often fail to translate into to the human.

P129

Genome-wide DNA methylation analysis of archival formalin-fixed paraffin-embedded tissue samples of squamous cell carcinoma

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Formalin fixation reveals excellent tissue quality for histopathological evaluation and is the method of choice for routine tissue preservation. However, in addition to morphologic analyses, molecular studies are more and more common to get further insights into disease pathogenesis and clinical outcome. Especially genetic and epigenetic analyses can be of great value for a better understanding of carcinogenesis. For such analyses formalin fixation has been reported to have a negative impact on the quality of DNA and RNA. While some large-scale methylation platforms can be used with formalin-fixed paraffin-embedded (FFPE) tissue, others are not suitable for FFPE tissue.

Here, we describe the analysis of FFPE samples of squamous cell carcinoma in comparison with the cryopreserved sample counterparts using a 450K BeadChip array. At least 500 ng of DNA of tumor cells was isolated from FFPE or cryopreserved samples and bisulfite modified. Over 485 000 methylation sites per sample could be analyzed at single-nucleotide resolution and revealed an excellent correlation between FFPE and cryopreserved samples ranging from $R^2 = 0.9544$ to $R^2 = 0.9876$.

Our results demonstrate the validity of data derived from FFPE samples in this array, which allows the application of epigenome-wide analyses of archival tissue specimens with valuable clinical follow-up information, particularly for studying rare conditions or specific variants of disease.

P130

Palmoplantar pustular psoriasis and its genetic background

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Palmoplantar pustular psoriasis (PPP) is a chronic inflammatory skin disease characterized by sterile pustules, erythema and hyperkeratosis on palms and soles. In at least 25% of PPP cases, psoriasis vulgaris (PsV) is also present and a significant proportion of patients suffers from psoriatic arthritis. Smoking and female sex are more frequent in PPP compared to PsV. So far, there are no confirmed genetic risk factors for PPP. Recently, in generalized pustular psoriasis, homozygous and compound-heterozygous mutations in the IL36RN gene have been identified to be causal. The same mutations have been described to be more frequent in a group of 139 PPP patients of European origin.

Here, we recruited a group of >140 PPP patients, most of them were female and smokers (>60%, respectively). About half of them had a manifestational age of Ü40 years.

We could confirm that the frequency of the HLA-C risk allele, the major genetic risk factor for PsV, was comparable to the frequency of controls indicating that PPP is genetically different from PsV.

We further analyzed IL36RN for mutations as well as for intragenic deletions and duplications and identified three heterozygous carriers of mutations and no carriers of copy number variants. Compared to a population-based control group of 4.300 European individuals, there was no significant difference in frequency of IL36RN mutations.

Our data indicate that PPP is genetically distinct both from PsV and generalized pustular psoriasis. Further effort is needed to identify genetic factors contributing to PPP.

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Compound heterozygosity for mutations in the coproporphyrinogen oxidase gene is associated with childhood-onset of severe blistering photosensitivity

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A partial deficiency of coproporphyrinogen oxidase (CPOX), the sixth enzyme in the heme biosynthetic pathway underlies hereditary coproporphyria (HCP). HCP belongs to the group of the acute hepatic porphyrias and is inherited in an autosomal dominant fashion. The disease does not usually manifest before puberty and is characterized by potentially life-threatening acute neurovisceral attacks and, rarely, by blistering photosensitivity on the sun-exposed areas of the body. We saw a 3-year-old girl who had developed severe photosensitivity, blistering and erosions on the hands and face since early childhood. Biochemical analysis showed highly elevated levels of uroporphyrin and coproporphyrin in the urine and a markedly increased fecal coproporphyrin III/coproporphyrin I ratio. Under the diagnosis of homozygous HCP, variegate porphyria or hepatoerythropoietic porphyria we initiated molecular genetic studies. Automated sequencing analysis of the CPOX gene revealed compound heterozygosity for two missense mutations, designated R328C and G333D, respectively, indicative of homozygous HCP. The causality of these mutations in disease pathogenesis was subsequently confirmed by prokaryotic expression of the mutant alleles. In infants and children with early-onset blistering photosensitivity the rare occurrence of a homozygous hepatic porphyria should be considered.

Immunology

P132

Dimethyl fumarate ameliorates psoriasiform dermatitis in the CD18hypo PL/J mouse model via combined effect on Treg/Th17 and γδ T cell plasticity

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Interleukin-17 (IL-17) producing Th17 and γδ T cells are critical players in the pathogenesis of psoriasis. According to the concept of T cell plasticity, differentiation of αβ and γδ T cell subsets towards a RORγthigh/Foxp3low/IL-17+ signature is significantly impacted by cell intrinsic epigenetic modifications as well as by environmental factors including oxygen supply and cytokine levels during inflammation. We herein investigated the influence of the anti-inflammatory and anti-psoriatic component dimethyl fumarate (DMF) on skin inflammation and differentiation of distinct IL-17 producing T cell subsets in CD18hypo PL/J mice, spontaneously developing psoriasiform dermatitis at 12–14 weeks of age as a consequence of reduced expression of CD18/β2 integrin to 2–16% of wildtype levels. Upon DMF treatment of affected CD18hypo PL/J mice for 8 weeks, significant improvement of clinical signs of skin disease was observed accompanied by a reduction of IL-17+ γδ T cells and Th17 cells with increased presence of Foxp3high+ T cells in lesional skin. In skin-draining lymph nodes of DMF-treated CD18hypo PL/J mice, total γδ T cell numbers remained elevated 8 weeks after treatment, but IL-7 mediated expansion of memory cells was reduced in culture and γδ T cells failed to upregulated the IL-23R. Notably, only minor changes in IL-23 producing CD11c+ dermal and bone marrow-derived dendritic cells were detectable in the CD18hypo PL/J psoriasis mouse model upon DMF application pointing towards a direct drug effect on T cell differentiation. At the molecular level, DMF action on expression and activity of hypoxia and redox sensitive transcription factors and signaling components, such as Hif1α, Nrf2 and Hemoxygenase-1 (HO1), and apoptotic pathways in αβ and γδ T cells was characterized. In a translational approach, DMF-treatment of αβ and γδ T cells expanded from human blood by IL-7 in vitro confirmed a stabilizing effect of DMF on Foxp3 levels and reduced differentiation towards IL-17 producing cells. In conclusion, DMF exerts a direct effect on the Foxp3/RORγt balance in αβ and γδ T cells via its influence on redox sensitive factors, Hif1α, Nrf2 and HO1, stabilizing regulatory properties of T cells. This newly identified mechanism of DMF action is likely crucial for the therapeutic efficacy of the drug in IL-17-mediated inflammatory diseases such as psoriasis and multiple sclerosis and provides novel insights into the redox dependence of αβ and γδ T cell plasticity.

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Molecules associated with T regulatory cell function are highly expressed on melanoma: novel targets for immunotherapeutic strategies

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Metastatic melanoma is the most frequent form of skin cancer-related deaths worldwide. Melanoma is a highly immunogenic tumor and many T cell epitopes have been described but most tumorantigen-reactive T cells are not sufficient. Especially regulatory T cells (Treg) and inhibitory factors of the tumor itself play a major role. Therefore we need a better understanding of molecules and pathways which regulate melanoma initiation and progression and further can be used as therapeutic targets or biomarkers. We investigated expression of well-known Treg-associated suppressor molecules using genome and proteome profiling of human Treg. We identified a distinct pattern of relevant Treg marker molecules and analyzed a panel of these makers in different human melanoma cell lines as well as in human melanocytes or primary melanoma in order to detect common molecules and pathways. Our results indicate that several marker molecules are highly expressed on melanoma cells compared to normal melanocytes. Surprisingly, the addition of IFN-α a well-known drug used as therapeutic adjuvant in melanoma patients decreases the expression of GARP (glycoprotein A repetitions predominant) on melanoma cells. GARP is an activation marker of Treg which has been shown to be involved in the mediation of immunosuppressive function and is highly expressed on melanoma cell surface. Thus, molecules shared by both melanoma and Treg might be hopeful targets for immunotherapeutic strategies in cancer patients. Importantly, the presence of GARP on melanoma cells reveals a new and functional relevant immunosuppressive mechanism in the tumor micro milieu.

P134

FcγRIIB, FcγRIII, and FcγRIV mediate tissue destruction in experimental bullous pemphigoid

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Bullous pemphigoid (BP) is a subepidermal autoimmune blistering disease mediated by autoantibodies against BP180. For blister formation, Fc-mediated events such as complement activation at the dermal-epidermal junction (DEJ), infiltration of inflammatory cells in the skin, and release of ROS and proteases at the DEJ are essential. While in the neonatal passive transfer mouse model of BP, Fcγ receptor (FcγR) I and III were shown to mediate tissue destruction, the passive transfer model of epidermolysis bullosa acquisita completely depended on FcγRIV. To clarify this discrepancy, we developed a novel experimental model for BP by the repeated injection of rabbit anti-murine BP180 IgG into adult mice. In this model, major clinical and immunopathological characteristics of the human disorder were reflected. As expected, lesion formation in this model was Fc-mediated since mice deficient for the common γ-chain of activating FcγR and mice treated with anti-BP180 IgG depleted from its sugar moiety at the Fc portion were resistant to the induction of BP. By the use of various FcγR-deficient mouse strains tissue destruction was shown to be mediated by FcγRIV and, to a lesser extent FcγRIII, while FcγRI not appeared to be essential and FcγRIIB was protective. The importance of FcγRIV was corroborated by its pharmacological inhibition which inhibited disease induction and even prevented disease progression in already clinically diseased mice. Here, we extended our knowledge about the importance of FcγRs in experimental BP and established a novel BP mouse model. In contrast to the neonatal model, it is suitable to study disease development over a longer time period and explore novel treatment strategies in a quasi therapeutic setting.

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Tolerogenic human IL-10-modulated dendritic cells: mature CD83high and immature CD83low DC subpopulations are inducers of potent regulatory T cells

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Human IL-10-modulated, tolerogenic dendritic cells (IL-10DC), which are capable to induce anergic regulatory CD4+ T cells (iTregs), consists of two subpopulations: mature CD83highCCR7highHLA-DRhigh and immature CD83lowCCR7negativeHLA-DRlow. Here, we investigated both IL-10DC subsets with regard to their phenotype and tolerogenic capacity in detail. As compared to fully mature DC (mDC) and the CD83high IL-10DC subset, the CD83low IL-10DC subpopulation exhibited a significantly diminished expression of the costimulatory molecules CD80, CD86, ICOS-L, and CD40. In contrast, on both IL-10DC subsets we observed a slight (CD83low IL-10DC) and significantly (CD83high IL-10DC) upregulation of the (co-)inhibitory molecules PD-L2, ILT3, and ILT4, demonstrating significant differences in expression of costimulatory and inhibitory molecules between the two IL-10DC subpopulations. Notably, primary stimulation of nave CD4positiveCD25lowCD45-RApositive T cells and restimulation experiments demonstrated that both IL-10DC subpopulations, regardless of their maturation state, induced anergic CD4+ T cells as evaluated by a significantly reduced T cell proliferation and IL-2 expression, associated with diminished Th1 and Th2 responses (reduced cytokine secretion and expression of transcription factors of T cell differentiation). In addition, both iTreg subpopulations showed regulatory properties and significantly suppressed the activity of responder T cells. The suppressive capacity of both IL-10 DC subsets was found after various stimuli (syngeneic mDC, anti-CD3/anti-CD28mAb, PBMC/anti-CD3mAb) and was accompanied by loss of their anergic state during suppressor experiments. In conclusion, mature CD83highCCR7highHLA-DRhigh and immature CD83lowCCR7negativeHLA-DRlow IL-10DC display properties of tolerogenic human DC, in particular as inducers of iTregs, which may be used as targets for the development of novel therapeutic approaches for allergies, autoimmune disease or transplant rejections.

P136 (O08)

Leukotriene B4/BLT1 act as gatekeeper of neutrophil recruitment into the skin in autoantibody-induced skin inflammation

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Recruitment of neutrophils into the skin is a hallmark of skin inflammation, but the molecular mechanisms mediating early neutrophil recruitment into the skin are still poorly understood.

We set out to elucidate the mechanisms of early neutrophil recruitment into the skin in organ-specific, autoantibody-induced skin inflammation using a mouse model of epidermolysis bullosa acquisita, a prototypical example of an autoimmune blistering skin disease caused by autoantibodies directed to collagen VII (COL7). Inflammation in this model is driven predominantly by neutrophils infiltrating the dermis.

We found that in this model early neutrophil recruitment into the skin absolutely depends on the lipid mediator leukotriene B4 (LTB4) and its receptor BLT1. Thus, deficiency in 5-lipoxgenase, a key enzyme in the biosynthesis of leukotrienes, or in BLT1, the high-affinity receptor for LTB4, conferred dramatic resistance to disease.

While wild-type mice developed severe clinical signs of EBA, including skin blistering, erythema, erosions, and crusts all over their body, and histopathologically displayed massive dermal infiltration predominantly with neutrophils, 5-lipoxygenase- (Alox5−/−) and BLT1-deficient (Ltb4r1−/−) mice did not develop signs of disease, neither clinically, nor histopathologically. Although anti-Col7 antibodies expectedly bound to basal membrane in these knockout mice, the skin, remarkably, remained devoid of neutrophils, suggesting a severe defect in the recruitment of neutrophils into the skin in Alox5−/− and Ltb4r1−/− mice.

Intradermal injection of exogenous LTB4 into Alox5−/− mice restored disease, indicating that LTB4 is the only leukotriene essential for autoantibody-induced skin inflammation. Similarly, adoptive transfer of either wild-type or Ltb4r1−/− neutrophils into the dermis of Ltb4r1−/− mice restored disease, albeit the latter did so to a lesser extent. These findings suggest that LTB4 is required to recruit neutrophils into the skin, but that LTB4 is dispensable for the activation of neutrophils within the skin.

We also examined the role of LTB4/BLT1 in imiquimod-induced psoriasiforme dermatitis (IIPD), another mouse model of sterile skin inflammation. In this model, skin inflammation closely resembling human plaque psoriasis is induced by epicutaneous application of imiquimod, a TLR7/8 agonist. In contrast to autoantibody-induced skin inflammation, deficiency in 5-lipoxygenase or BLT1 did not significantly modulate the course of TLR7/8 activation triggered skin inflammation, revealing that dependency of skin inflammation on LTB4/BLT1 is apparently stimulus-specific.

Altogether, our results hint at a role of LTB4/BLT1 as critical gatekeeper of neutrophil egress into the skin in organ-specific, autoantibody-induced inflammation. LTB4/BLT1 are hence promising pharmacological targets for the treatment of autoimmune blistering skin diseases.

P137

MPI-2 cells, a new model for tissue type macrophages

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Macrophages are diverse cell types in the first line of anti-microbial defense and, depending on the subset, they can promote or dampen inflammation. So far, mouse bone marrow-derived, M-CSF-induced macrophages (BMM) are the most common model to study macrophage functions. However, BMM have limited lifespan and represent only a particular subset of macrophages. We developed a simple method yielding self-renewing, non-transformed, GM-CSF/STAT5-dependent macrophages (MPI cells) from mouse fetal liver. Unlike other types of primary macrophages, MPI cells from various wild type, gene-deficient or transgenic mice can be propagated indefinitely in unlimited quantities and can be easily manipulated genetically.

MPI cells are sensitive to selected microbial agents, including LPS, lipopeptide, Mycobacterium tuberculosis, cord factor and adenovirus, and show a pattern of innate responses to these stimuli that is similar to lung alveolar macrophages.

In the skin, macrophages are involved in immune surveillance and wound repair, but they play a crucial role in several inflammatory skin diseases as well. Similar to dermal macrophages, MPI cells are only weakly positive for CD11c and MHCII, but express Dectin-1. The establishment of MPI cells as model for macrophage responses in the skin is still in progress. First results show, that MPI cells are able to efficiently phagocytose Propionibacterium acnes, a constituent of the skin flora, and produce pro-inflammatory cytokines like IL-6 and IL-1.

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Recombinant human IgA1 and IgA2 autoantibodies to type VII collagen induce subepidermal blistering ex vivo

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Subepidermal autoimmune blistering dermatoses (AIBD) are prototypicorgan-specific autoimune diseases, characterized by autoantibodies directed to structural proteins of the skin. In epidermolysis bullosa acquisita (EBA), an AIBD with autoantibodies directed to type VII collagen (COL7) IgG is the predominant isotype of autoantibodies, and their pathogenicity has been demonstrated independently by use of in vivo and ex vivo disease models. Furthermore, in anti-COL7 IgG induced model systems, activation of complement has been identified as a crucial step to initiate blister formation. In contrast, potential of IgA autoantibodies to induce blistering in EBA has not been elucidated in detail. IgA is the only isotype of autoantibodies detected in approximately 25% of EBA patients. To evaluate the pathogenic potential of IgA, we generated chimeric V gene-matched human IgA1,IgA2 and IgG1 autoantibodies directed against COL7. Immobilized immune complexes containing recombinant IgA1 and IgA2 autoantibodies induced dose-dependent release of reactive oxygen species (ROS) from neutrophilgranulocytes, a precondition for blister formation. Moreover, both IgA1 and IgA2 autoantibodies induced leukocyte-dependent dermal-epidermal separation in cryosections of human skin. In contrast to IgG1, neither IgA1 nor IgA2 were capable to induce complement deposition at the dermal-epidermal junction. As complement activation is aprerequisite for blister induction, this lack of function compared to IgG1 might be compensated by stronger activation of neutrophil granulocytes by both IgA1 andIgA2. The results of this study should encourage the development novel treatment modalities like immunadsorption therapy. Immunoadsorption therapy is a convenient method for removal of pathogenic IgG autoantibodies in treatment resistant AIBD, but is currently not available for IgA-mediated diseases.

P139

Regulatory T cell – deficient scurfy mice develop systemic lupus-like disease

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Introduction: Systemic lupus erythematodes (SLE) is a severe systemic autoimmune disease with involvement of skin and inner organs. Autoreactive (CD4+) T cells, B cells and autoantibodies are crucial for the pathophysiology of Lupus. CD4+FoxP3+ regulatory T cells (Treg), on the other hand, are important for maintaining peripheral tolerance to self-antigens. Several studies described that both, the absolute numbers and the functionality of Treg are reduced and inversely correlate with disease activity, but direct evidence for a central role of Treg dysfunction in SLE pathophysiology is still missing. We therefore analyzed, if Treg-deficient scurfy mice share typical features of SLE.

Methods: Scurfy mice lack functional Treg due to a genetic defect in the transcription factor foxP3, which is crucial for Treg development and function. 9 scurfy and 9 matched controls (C57Bl/6 mice) were analyzed at 4–5 weeks of age: By immunofluorescence, immunoblotting and ELISA, we screened for autoantibodies and also performed hematological workup. Specimen of skin and inner organs were stained with H/E and screened for inflammation by a blinded pathologist; kidneys were also stained with PAS and by direct immunofluorescence. We analyzed joint pathology after staining with H/E (overview), tolloidin blue (cartilage) and TRAP (osteoclasts). Immunhistochemistry allowed for further analysis of the cellular composition of the inflammatory infiltrate, which was finally quantified by image analysis systems (Osteomeasure and HistoQuest, respectively).

Results: We confirmed previous reports that scurfy mice spontaneously develop severe systemic autoimmune disease which includes pneumonitis and hematological abnormalities similar to those seen in SLE.

In addition we show that scurfy, but not WT control mice, exhibit various additional features typical for SLE: They tested positive for ANA (100%) as well as for anti-dsDNA-abs (100%), anti-Sm (80%), anti-RNP (90%) (but not for anti-Ro, -La, or -Scl70-abs; immunoblot) and had elevated anti-histone-ab levels (14 558 vs 6636 U/ml, $P = 0.003$, ELISA); scurfy also developed severe interface dermatitis and mesangial glomerulonephritis resembling lupus nephritis WHO2 (seen in 8 out of 9 scurfy [88.9%] vs 0/6 [0%] of controls, respectively, $P = 0.0014$). In contrast to controls, scurfy mice showed increased cartilage degradation (destained/normal cartilage area 0.0500.009 vs 0.0180.003, $P = 0.004$) and developed inflammatory arthritic infiltrates (mean area 0.380.25 mm2). There were no osteoclasts within the joint space and, consecutively, no erosions. Besides fibroblasts, the inflammatory infiltrate consisted mainly of CD3+ T lymphocytes (13%), with 7% B cells and <3% neutrophils and macrophages. In transfer experiments, CD4+ T cells from scurfy, but not from controls, induced production of ANA and dsDNA-abs in athymic nude mice.

Conclusion: Our observations support the hypothesis that absence of functional Treg induces lupus-like disease.

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Langerhans cells with selective deficiency of manganese superoxide dismutase have features of LC in aged skin

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The aged immune system is characterized by impaired antigen specific immunity but coexistent susceptibility to autoimmune disease. LC are a special subset of dendritic cells that are located within the epidermis. Depending upon the context of their activation Langerhans cells (LC) seem to fulfill antigen presenting functions both in tolerance induction and antigen specific immune response. Aging has been linked to deregulation of radical oxygen species (ROS) and is associated with a reduction in the number of LC. The mitochondrial manganese superoxide dismutase (SOD2) is an important helper in antioxidant defence, which is supported by the finding that mice with conditional deficiency for SOD2 in the connective tissue show an accelerated aging phenotype.

We sought to obtain insights as to whether SOD2 plays a role in an impaired function of LC in the aging skin immune system. To this end, mice transgenic for a bacterial artificial chromosome containing the gene for human Langerin into which Cre recombinase had been inserted (Langerin-Cre) were bred to floxed SOD2 mice, to generate mice with a deficiency of SOD2 selectively in epidermal LC (Langerin-Cre SOD2f/f mice). To test the implication of SOD2 deficiency on ROS balance isolated LC from Langerin-Cre SOD2f/f mice were treated with pyocyanin, a strong inducer of oxidative stress. Upon challenge, such LC contained high amounts of superoxide anion but less other ROS which is explained by the missing transformation of superoxide into hydrogen peroxide and diatomic oxygen by SOD2. We went on to investigate the effects superoxide accumulation in LC by staining LC in ear skin sheets of 6 to 8-week-old mice and found that LC in Langerin-Cre SOD2f/f mice were smaller and had lost their dendritic morphology. This was further supported by FACS

analysis of epidermal cell suspensions that revealed that Langerin+ cells were reduced up to 25% in skin of these mice. Investigating the expression of cell surface markers on LC we found that SOD2 deficient LC expressed slightly less MHC-II and CD86. However, LC were not compromised in their antigen uptake capacity as determined by normal FITC-dextran uptake. Their antigen presenting function was not altered as they had normal T cell stimulatory capacity in MLR. Finally, to determine how SOD2 deficiency of LC would influence contact hypersensitivity (CHS) response, mice were sensitized against TNCB. Upon challenge, Langerin-Cre SOD2f/f mice had a significantly inhibited CHS response 48 h after TNCB challenge. Our findings indicate that superoxide toxicity due to the loss of SOD2 function even in young mice leads to fewer LC with morphologic changes eventually leading to mild impairment of CHS in vivo. These are all features observed for LC in aged skin.

P141 (O28)

ADV-specific γ/δ and CD8+ T cells generated by TCR-RNA electroporation for the treatment of adenovirus infection after allogeneic stem cell transplantation; new therapeutic possibilities

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The last resort in treating chemotherapy-resistant hematopoietic malignancies in children can be an allogeneic haematopoietic stem cell transplant (HSCT) combined with chemo-and radiotherapy. The time to immunological engraftment of the transplant can take up to several weeks or even months. There is often no protection against adenovirus (Ad) infection during this time, which causes substantial transplant-associated mortality. The use of antiviral drugs is limited and associated with significant side effects and results in prophylactic overtreatment of many patients. The adoptive transfer of Ad-specific T cells is an alternative therapy. However, 10 to 20% of all HSCT donors lack Ad-specific T cells. Here, the solution could be to equip donor T cells with a specificity for Ad by TCR transfer.

As a proof of principle, we transfected CD8+ T cells with mRNA encoding an HLA-A1-restricted, Ad-specific TCR. These cells specifically recognized HLA-A1+ dendritic cells (DC) as well as tumor cells loaded with the corresponding LTDLGQNLLY peptide and responded with secretion of the pro-inflammatory cytokines IL-2, TNF, and IFNγ. They were also able to lyse peptide-loaded target cells. Most importantly, Ad-infected target cells were also recognized by TCR-transfected CD8+ T cells in an antigen-specific manner.

As reprogrammed T cells still express their endogenous TCR, they may be alloreactive and might cause graft versus host disease (GvHD). γ/δ T cells, in contrast, are in general not alloreactive, and hence would be good tools to circumvent alloreactivity against recipient cells if the donor is not completely matched.

To improve safety, we electroporated $\alpha\beta$-TCR-mRNA into γ/δ T cells. These reprogrammed cells produced low quantities of IL-2, TNF, and IFNγ in response to peptide-loaded target cells. Since most γ/δ T cells are CD8-negative, and the introduced TCR might benefit from CD8 co-binding, we therefore co-electroporated CD8-encoding mRNA. These TCR-CD8-co-transfected γ/δ T cells showed an increased cytokine production, and, moreover, in a direct comparison with TCR-transfected CD8+ α/β T cells, they produced more TNF and IFNγ in response to the antigen. In addition, these TCR-transfected γ/δ T cells lysed peptide-loaded target cells efficiently.

Taken together, we show here for the first time that not only α/β T cells but also γ/δ T cells can be endowed with a specificity for Ad by TCR-RNA electroporation. Thus, our strategy offers a new means for the immunotherapy of Ad infection after HSCT.

P142

Merkel cell carcinoma as a model for immunotherapy of virally induced cancer by vaccination against the large T antigen

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Cancer is one of the major causes of death worldwide. In about 15% of human cancers, viruses are involved. One cancer that comprises a viral involvement is the Merkel Cell Carcinoma (MCC). MCC is a neuroendocrine skin tumor, which is relatively rare, but highly aggressive and its incidence rates increased over the last decades. Aside from surgical excision no standard treatments exist for already established tumors. Most recently the Merkel Cell Polyomavirus (MCV) was discovered to be associated with MCC (80% of MCC tissues contain MCV, but only 8% of healthy tissues). Even though MCV is common in the healthy population, the virus can, under certain circumstances, integrate into the host genome. Thereby, the production of most viral proteins is switched off, but a truncated form of one of its viral proteins, the large T antigen (LT) is still expressed. This protein contains conserved tumor suppressor-targeting motifs, e.g. the Rb binding domain, and serves as oncogenic driver. Moreover, LT is a foreign antigen and similar in a large number of patients. Thus, LT seems to be an appropriate target for the immunotherapy of cancer.

So far immunotherapy of cancer mostly used self-antigens that are only over-expressed on cancer tissue. This requires a breach of central tolerance and may also confer the risk of establishing autoimmunity against healthy tissue. An ideal target antigen would be a foreign antigen that can be recognized by the immune system more efficiently. Hence, the aim of this study is to develop an immunotherapeutic DC vaccination approach for the treatment of virally induced cancers by employing MCC with its LT as a model disease. We intend to induce long-lived memory CD8+ T cells, which recognize the LT in an antigen-specific manner, by priming with designer dendritic cells (DC). These DC will be generated by the electroporation with truncated LT (truncLT)-RNA. To improve the DC's immunogenicity, we will co-transfect constitutively active mutants of IκB kinases (caIKK) of the NF-κB signaling pathway. We have already shown that the introduction of caIKK, i.e. caIKKα and caIKKβ, led to the up-regulation of maturation markers and co-stimulatory molecules. Besides, also an increase of cytokine secretion, mainly TNF, IL-6, IL-8, and IL-12p70 was detectable. In concert, the increased expression of co-stimulatory molecules and a beneficial IL-12p70 / IL-10 ratio made these DC highly immunogenic and potent stimulators for the generation of antigen-specific memory-like CD8+ T cells. To further improve the DC's stimulatory capacity, we codon-optimized the caIKK constructs and compared the DC transfected with the optimized and standard constructs concerning surface expression of DC maturation markers and co-stimulatory molecules. Additionally, cytokine secretion was assessed in a time-dependent manner. Accordingly, since the cytokine production capability and the stimulatory potential of the DC clearly changed by using the optimized constructs, we design the ongoing study either with the optimized or the standard constructs after further corroboration of the preliminary results. After having further improved DC immunogenicity, we plan to determine whether the co-electroporation with truncLT and caIKK results in designer DC capable of inducing LT-specific T cells, which efficiently kill MCC cells. These generated LT-specific T cells will then be further characterized and compared to patients' LT-specific T cells. Thus, we will assess whether the LT is a suitable target antigen for the DC vaccination therapy of MCC.

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Transfection of mRNA into human mature DC does not change their transcriptional program

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Dendritic cells have emerged as a favorite tool in cellular immunotherapy in the last decades. For therapeutic cancer vaccination, the adoptive transfer of antigen (Ag)-loaded DC, is now frequently performed, usually with monocyte-derived DC (Mo-DC) loaded with tumor Ag, because these cells are easily accessible and well-characterized.

An attractive method to load these DC with Ag, is the transfection with mRNA. However, as the natural function of DC is to recognize pathogen-derived structures and danger signals, they are rich in danger-sensing receptors such as the Toll-like receptor (TLR) family. It was reported that exogenously delivered mRNA could induce DC activation through such mechanisms. This DC activation resulted in the up-regulation of activation markers and cytokine production, which could have important implications for DC-based immunotherapy.

Therefore, we examined whether electroporation with mRNA that was produced in compliance to GMP, and was properly capped and contains a poly-A tail of at least 64 A has an influence on cocktail-matured Mo-DC. We used 15 different RNAs, encoding different tumor antigens (e.g. NRAS, BRAF, GNAQ, GNA11, and WT1), either mutated or not, and either linked to the lysosomal targeting signal DC-Lamp or not. None of those RNAs induced changes in the expression of CD25, CD40, CD83, CD86, and CD70 of more than 1.5 fold, or increased the secretion of the cytokines IL-8, IL-6, and TNF to more than 130% of the control condition.

Furthermore, we performed microarray analyses to explore in more detail whether mRNA electroporation had any effect on the whole transcriptome of the DC. Only four probes out of 60 000 were found to be significantly different between mock-electroporated DC and MelanA-transfected DC: of these, two mapped to different regions of the same gene, resulting in 3 unique differentially expressed genes (DEGs) (i.e., interferon-induced protein with tetratricopeptide repeats 3, interferon-induced protein 44, XIAP associated factor 1). As the number of the differentially expressed genes was extremely small, we conclude that no transcriptional programs are induced within cocktail-matured DC by electroporation of single mRNAs.

In summary, these data show that in our case the introduction of mRNA into human cocktail-matured Mo-DC by electroporation does neither result in a difference in phenotype, nor in cytokine production, nor in substantial changes in the transcriptome of these cells.

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Tumor infiltrating B-cells in primary cutaneous T-cell lymphoma correlate with disease progression and might represent a novel target for immunotherapy

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Mycosis fungoides (MF) and other primary cutaneous T-cell lymphomas (CTCL) are characterized by an indolent course in early stages. However, advanced stage MF (? EORTC stage IIB) and the follicular MF subtype (FMF) as well as S©zary syndrome (SS) show a more aggressive pattern with a median survival of <2 years. The pathogenesis of these aggressive courses is still incompletely understood.

B cells have been recently described to mediate tumor biology but so far their role as a tumor promoting or tumor repressing bystander population remains controversial.

With regard to a potential role of tumor associated B cells in CTCL, we systematically analyzed the B-cell infiltrate in tumor samples of CTCL patients ($n = 33$) by immunohistochemistry (CD20 and CD79a) and, if possible, by flow cytometry. These data were correlated with the stage and clinical course of the disease. Non-malignant T-cell mediated skin diseases (psoriasis and ekzema; $n = 10$) served as controls.

Advanced stage MF and all FMF and SS samples showed significantly increased B-cell infiltrates per lymphoma tissue. Moreover, time to progression showed a significant inverse relationship with the density of the B-cell infiltrate.

Based on these results, we hypothesized that infiltrating B cells might promote lymphoma progression and could therefore serve as a therapeutic target. In a 77-year old patient suffering from advanced stage FMF with a significant B-cell infiltration and progression after standard treatments, B-cell depletion with the anti-CD20 monoclonal antibody rituximab resulted in a sustained complete local tumor regression. More interestingly, B-cell depletion led to a remodeling of the immune infiltrate, i.e. re-established a cytotoxic T-cellular anti-tumor response.

In summary, we present first evidence for the potential tumor promoting role of CTCL associated B cells which warrants further study as a potential therapeutic strategy.EUR,

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The influence of binding affinity and receptor density of chimeric antigen receptors on target-cell recognition after transfer by RNA electroporation

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During the last decade chimeric antigen receptors (CARs) have become a potent and promising tool in the immunotherapy of cancer. Although CAR-transduced T cells showed strong anti-tumor responses in cancer patients, severe side-effects occurred in several patients. These side effects were due to an on-target, off-tissue reaction of the transduced T cells, as most CARs target self-antigens, which are overexpressed in tumor tissues, but can also be found on healthy tissue.

Therefore it is necessary to enable the T cells to discriminate between high and low antigen levels on tissues and tumor cells. We thus wanted to explore the influence of CAR affinity and CAR density on the target-cell recognition.

To fully investigate these issues it is crucial to adjust the surface density of the CAR molecules to desired levels, to allow for a direct comparison of CARs with a different affinity to the target antigen. In contrast to retroviral transduction, where CAR expression levels are difficult to manipulate, the electroporation of CAR-encoding mRNA allows to directly influence CAR-expression density by adjusting the amount of transfected mRNA.

In this study we efficiently electroporated T cells from healthy donors with mRNA encoding different CAR-molecules, which target the same antigen with different affinities. We show that the amount of RNA used during electroporation directly impacts CAR-expression levels and that the receptor density on the T-cell surface can be adjusted to identical levels for different receptors. This enabled us to investigate the influence of CAR affinity and CAR density on the recognition of cells with high or low antigen levels. We explored the cytokine secretion profiles and cytolytic capacity of the

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CAR-transfected T cells in regard to CAR density and binding affinity after stimulation with tumor cell lines with different levels of the target antigen.
Taken together we provide a tool to investigate the function of CAR molecules targeting the same antigen with respect to antigen affinity and receptor density. The method allows for the selection of CARs for a potential clinical application by lowering the risk of unwanted T-cell activation upon recognition of low-level antigen expression on healthy tissue.

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IFN-α inhibits human FOXP3+ regulatory T cells through ERK-and PDE4-dependent cAMP repression

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INF-α induces autoimmune symptoms in cytokine-treated cancer patients and is critically involved in autoimmune diseases like systemic Lupus erythematosus, suggesting an effect of the type I interferon on tolerance processes. Recently, we showed that IFN- abolishes the suppressor activity of human CD4+CD25high FOXP3+ regulatory T cells (Treg) in vitro and in vivo in a humanized xenogenic GVHD mouse model. Disarming of Tregs by IFN-α was accompanied by a repression of intracellular cAMP. Therefore, this study focussed on IFN-α-induced intracellular signalling with respect to cAMP regulation. As cAMP levels are controlled by phosphodiesterases-mediated degradation, we used the non-selective phosphodiesterase inhibitor IBMX and the PDE4 (highly expressed in T Cells) specific inhibitor rolipram. Both inhibitors restored cAMP amounts and therefore renewed the suppressive function of human Tregs. This emphasizes the functional relevance of IFN-α-induced reduction of cAMP in Treg. Activation of PDE4 in T cells is controlled by the MAP kinases Erk1/2. Blocking of this pathway by an Erk-specific inhibitor in IFN-α-treated Treg completely restored cAMP accumulation, indicating that IFN-α repressed cAMP levels through modulation of Erk pathways that subsequently regulated PDE4 activity. However, we did neither observe an alteration of the anergic state, of the cytokine profile nor of the methylation status of the Treg-specific-demethylated region (TSDR) within the Foxp3 locus that have been shown to be crucial for the stability and function of the FOXP3 promoter. Thus, we found that IFN-α did not modulate the Treg differentiation program. In conclusion, this study demonstrates that IFN-α interferes with the suppressive activity of human CD4+CD25+FOXP+ Treg by affecting cAMP regulation through MAP kinase/PDE4-mediated pathways, suggesting a transient Treg inhibition as an important mechanism in IFN-α-mediated immune regulation.

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The phagocyte NADPH oxidase NOX2 controls physiological wound healing

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Leukocytes play an essential role in the inflammatory phase of the wound healing process. We have previously shown that beta2 integrin leukocyte adhesion molecules and their downstream signaling targets Vav3 and Rac2 causally control leukocyte functions during wound healing, especially the phagocytosis of apoptotic neutrophils (PMN) by macrophages (Mf). Engulfment of apoptotic PMN results in the release of reactive oxygen species (ROS) and active TGF-beta1 by Mf and, thus, is indispensable for normal wound healing. The enzyme mainly generating ROS in phagocytes is the NADPH oxidase NOX2, with Rac2 representing one of its 6 subunits.
The purpose of this project was to investigate whether insufficient ROS production in NOX2 deficient conditions would lead to reduced release of active TGF-beta1 at wound sites and to impaired wound healing. We first performed in vivo imaging experiments with p40phox−/− mice which lack p40phox, a cytosolic subunit of NOX2, resulting in severely impaired NOX2 function in phagocytes. P40phox−/− mice injected with the redox-sensitive chemiluminiscent probe L-012 as substrate mounted significantly reduced levels of ROS at wound sites when compared to wildtype (WT) control mice. Notably, in a model of full thickness excisional wounds, p40phox−/− mice displayed a significantly delayed wound healing compared to WT mice. Immunofluorescence stainings on wound cryosections for alphaSMA and CD31 revealed markedly impaired granulation tissue formation in p40phox−/− wounds compared to WT wounds. These findings were further supported by immunoblotting of p40phox−/− and WT wound tissue lysates for TGF-beta receptor II and alphaSMA. Both granulation tissue markers were significantly reduced in wounds of NOX2 deficient mice compared to WT wounds. Assessment of active and total TGF-beta1 levels in wound lysates further showed significantly reduced levels of active TGF-beta in 5 and 7 days old wounds of p40phox−/− mice compared to WT control mice. These data strongly suggest that in p40phox deficiency reduced ROS levels result in delayed granulation tissue formation and impaired wound healing, very much resembling defective wound healing in beta2 integrin deficient conditions which is driven by improperly functioning Mf.
To investigate whether NOX2 is a downstream target of beta2 integrins in Mf during wound healing we injected WT or p40phox−/− Mf around wound margins of full-thickness excisional wounds in beta2 integrin (CD18)−/− mice which lack functional beta2 integrins due to mutations in their common beta subunit CD18. Injection of p40phox−/− Mf in CD18−/− wound margins failed to improve the impaired wound healing of CD18−/− mice at any time point, while injection of WT Mf fully rescued this healing defect. These findings suggest NOX2 to be a downstream target of beta2 integrins in Mf during wound healing.
Taken together our results show that NOX2 critically controls physiological wound healing and that NOX2 activation during wound healing, at least in phagocytes, is beta2 integrin dependent. Impaired NOX2 activation leads to reduced oxidative burst at wound sites, reduced activation of TGF-beta1, impaired granulation tissue formation and eventually in impaired wound healing. The modulation of NOX2 activation in Mf and thereby ROS levels at wound sites may provide promising therapeutic strategies for Mf-driven inflammatory disorders.

P148

Thymic stromal lymphopoietin (TSLP) function as a potential differentiation factor is upregulated to bias strong Th2-polarization in Scurfy inflamed skin and lung

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Scurfy mice have a deletion in the Foxp3 gene, which results in the lack of functional Foxp3+ regulatory T cells, and they subsequently develop severe autoimmune multiorgan inflammation including skin and lung. It is known that autoreactive CD4+ effector T cells mediate the disease since isolated Scurfy CD4+ T cells transfer the same disease in RAG−/− recipients after i.v. injection.
Thymic Stromal Lymphopoietin (TSLP) is secreted primarily by epithelial cells and characterized as a lymphocyte growth factor. Recent studies have shown that TSLP, acting on CD4+ T cells and dendritic cells, can promote Th2 cell differentiation and Th2 cytokine-associated immune response.
In this study, we analyzed the inflammatory infiltrate of Scurfy skin and lung and the cytokine profile of skin-infiltrating autoreactive CD4+ T cells especially in regard to TSLP as potential differentiation factor.
CD4+ T cells and granulocytes are the predominant cell types in inflamed skin by FACS-analysis. When we used intracellular FACS-analysis after in vivo restimulation with PMA/Ionomycin, We observed CD4+ T cells isolated from inflamed Scurfy skin expressed high levels of Th2-cytokines (IL-4 and IL-5), but low levels of the Th1-cytokine interferon-γ. Since TSLP is known to mediate Th2-differenciation of CD4+ T cells, we analyzed the expression of TSLP. We found that Scurfy mice presented high TSLP-serum levels compared to WT mice as measured by ELISA. To identify TSLP gene expression in Scurfy skin and lung, we performed RT-PCR analysis and found upregulated expression of TSLP RNA in Scurfy but not WT lung and skin epithelial cells. We also analyzed TSLP expression by immunohistochemistry in inflamed Scurfy skin and lung and demonstrated high TSLP expression in Scurfy epidermis and lung but low expression in WT mouse tissues. Finally, to determine if skin-infiltrating CD4+ T cells can respond to TSLP, we analyzed TSLP-receptor-expression by FACS-analysis. The result demonstrated that CD4+ T cells in Scurfy but not in WT skin showed upregulated TSLP-receptor expression.
Taken together, these data indicate that Scurfy autoreactive CD4+ T cells spontaneously develop a Th2-phenotype with high expression of the Th2-differentiation factor TSLP in Scurfy skin and lung and serum as potential driving factor for this Th2-polarization.

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Diabetes mellitus type I protects from allergic contact dermatitis in mice

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Among allergic skin disorders, the allergic contact dermatitis (ACD) accounts for a high percentage of all dermatological consultations, and leads to considerable impairment of live quality. ACD is a CD8+ Tc1 T cell-mediated inflammation of the skin which occurs after re-exposure to the offending hapten. Currently, preventive and therapeutic strategies are avoidance of the allergen or treatment of clinical symptoms. However, epidemiological studies noted a lower prevalence of ACD in individuals with autoimmune diseases like type I diabetes as compared to healthy individuals. In our study, we investigated the influence of the development of type I diabetes on the outcome of a contact hypersensitivity reaction (CHS), the mouse model of human ACD. For this purpose, the murine non-obese diabetes (NOD) model was used in which mice spontaneously develop an autoimmune insulin-dependent diabetes mellitus, resembling the human type I diabetes. Blood glucose levels of female NOD mice were monitored and considered diabetic after two consecutive readings above 250 mg/dl. In order to induce a CHS, the mice were epicutaneously sensitized with a contact sensitizer (e.g. the hapten TNCB), followed by an application of the hapten onto the ear to elucidate the CD8+ Tc1-mediated skin inflammation. We compared the impact of the diabetic phenotype on the development of the CHS reaction in diabetic versus non-diabetic NOD mice. Notably, the existence of a clinically apparent diabetes in NOD mice protected from a CHS reaction as demonstrated by a significantly reduced skin inflammation (diminished ear swelling) as compared to non-diabetic NOD mice. In addition, an impaired hapten-specific T cell-proliferation and reduced Tc1-cytokine pattern (IFN-γ, IL-2) production was observed in diabetic mice, indicating an attenuation of the development and severity of a CHS reaction by a diabetic phenotype. In summary, this study demonstrated that the manifestation of an autoimmune disease like type I diabetes mellitus prevents from the development of an allergic CD8+ Tc1-mediated skin inflammation (CHS) in mice and, therefore, confirmed the data of a reduced incidence of allergic contact dermatitis in patients suffering from diabetes and other autoimmune diseases. The identification of underlying immune mechanisms may identify a novel link between allergy and autoimmunity and may result in innovative therapeutic and preventive strategies for allergic and autoimmune diseases.

P150 (O31)

Immune suppression in severe atopic dermatitis is mediated by myeloid derived suppressor cells

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The skin harbors an active immune network playing a crucial role in host defense and in shaping immune responses. We aimed to investigate how the constant interaction with Gram-positive Staphylococci impacts the immune system. Atopic dermatitis (AD) is a chronic inflammatory skin disease that is nearly always covered with and triggered by Gram-positive Staphylococci and thus serves as a human model for studying the immune consequences of skin activation by bacterial components.
Surprisingly, using mouse models we observed a strong immune suppression after cutaneous exposure to TLR2 ligands and living *S. aureus*. This immune suppression was mediated by myeloid derived suppressor cells (MDSCs). Using the appropriate knock-out and chimeric mice, we further identified that TLR2 on skin resident cells and induction of IL-6 are responsible for MDSC induction, MDSC recruitment to mouse skin, and T-cell suppression. To take our findings further, we next analyzed AD patients, in which cutanoues TLR2 is constantly activated by Staphylococci derived substances. In humans MDSCs are typically described as CD11b+ CD33 + HLA-DR-CD14- cells and we observed a significant increase of MDSCs in the peripheral blood of AD patients ($n = 33$) in comparison to healthy individuals ($n = 30$). Further investigations of peripheral blood mononuclear blood cells (PBMCs) revealed a significant down-regulation of T-cell receptor -chain in AD patients, which is known to be a general characteristic of immune suppression and one of the major features of MDSCs-mediated T-cell inhibition. To further investigate whether human MDSCs were suppressive, we depleted CD11b+ cells from PBMCs and indeed found T-cell proliferation to be increased in AD patients compared to controls. This finding demonstrates that MDSCs, which are present among the CD11b+ population in AD patients but not in healthy individuals, are immunosuppressive. Nitric oxide (NO) is one of the main suppressive factors produced by MDSCs. The inducible NO-synthase (iNOS) and arginase can generate NO from L-arginine. Indeed we found significantly elevated arginase activity in plasma of AD patients and we detected a distinct iNOS+ population of CD11b+ CD11c- cells among PBMCs in AD patients. These cells were completely absent in healthy individuals. To investigate whether MDSCs accumulate in the skin we next performed FACS analysis of human skin and found CD11b+ CD33+ HLA-DR-CD14- cells significantly elevated in AD skin compared to controls, especially prominent in eczema herpeticum. Moreover, using three colour fluorescence immunohistology in AD skin samples we detected iNOS+ CD11b+ CD11c- cells. These date indicate that MDSCs are not only increased in AD blood and skin, but also exert their suppressive activity. Interestingly, a severe complication of AD is eczema herpeticum which is characterized by spreading cutaneous infections with herpes simplex virus (HSV) due to immune suppression. Thus, we hypothesize that severe bacterial colonization in AD and subsequent skin inflammation causes induction of suppressive MDSCs, which then accumulate in the skin and exert their suppressive activity allowing e.g. herpes viruses to spread.
Our findings demonstrate a new level of immune regulation orchestrated by the skin and indicate that MDSCs can be diagnostic markers and therapeutic targets in severe disease.

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Sulfated fragments of hyaluronan suppress inflammatory macrophage activation

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Hyaluronan (HA) is an essential component of the extracellular matrix (ECM) that is known to excert pro- or anti-inflammatory effects depending on its size. While high molecular weight (HMW) HA downregulates and protects from inflammation and thus promotes homeostasis, low molecular weight (LMW) HA rather elicits pro-inflammatory responses by activating immune cells. Our group focusses on the development on immunomodulating biomaterials based on artificial ECM (aECM) that may be used as wound dressing for non-healing wounds. These chronic wounds are typically characterized by a persistent inflammation driven by unopposed M1 macrophage activation. In this respect we have recently shown that aECM composed of collagen I and sulfated hyaluronan possesses promising immunomodulating properties as it downregulates inflammatory M1 macrophage functions by impeding inflammatory gene transcription and cytokine productions. Here we demonstrate that this anti-inflammatory effect is mediated by the sulfated hyaluronan which has a size of 50 kDa and is thus classified as LMW-HA fragment. We therefore questioned whether chemical modification with sulfate groups endows HA with anti-inflammatory properties which prevails the pro-inflammatory size effect. To address this we assessed the effect of HMW-HA (1174 kDa), LMW-HA (50 kDa) and a sulfated derivative of LMW-HA (50 kDa) on functions and phenotype of inflammatory M1 macrophages. We found that HMW-HA as well as sulfated LMW-HA suppress inflammatory activation of macrophages whereas LMW-HA had no impact on the macrophage response. In the presence of both HMW-HA and sulfated LMW-HA release of the inflammatory cytokines TNF, IL-12, IL-23 and their gene transcription was attenuated due to impaired NFkB activation. Interestingly, although both HMW-HA and sulfated LMW-HA engage with the intracellular downstream signalling pathway of inflammatory gene activation they perform their anti-inflammatory effect in a different kinetic. While HMW-HA immediately suppresses inflammatory cytokine production sulfated LMW-HA significantly exerts this effect only after three days macrophage culture. Moreover, titrating the lowest effect concentration we observed that sulfated LMW-HA shows its anti-inflammatory impact on macrophages at concentrations at which HMW-HA is already ineffective.

In summary our data show that sulfation of LMW-HA generates HA fragments with anti-inflammatory properties. Mechanisms by which sulfated LMW-HA initiate its anti-inflammatory effect in the cells seems to be different from those of HMW-HA and are currently further investigated. Additionally we assess whether sulfated LMW-HA downregulates inflammatory functions of other important cells of the healing response.

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Passive transfer of type VII collagen-specific autoantibodies into mice induces not only skin lesions but also germinal centers

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Epidermolysis bullosa acquisita (EBA) is an autoimmune bullous dermatosis (AIBD), characterized by autoantibodies against type VII collagen (COL7), a structural protein of the skin. Skin blistering mirroring the findings in EBA patients can be induced by passive transfer of antibodies specific to COL7 into mice. In this model, anti-COL7-specific IgG from rabbits was injected i.p or s.c. into C57BL/6 mice. 4–6 days later, skin lesions appear mainly on friction-exposed sites like ears and eyes. In contrast, local injection of anti-COL7-specific IgG subcutaneously intradermally into the ear induced robust skin lesions within 1–2 days. The reason for this striking difference in the course of the pathophysiology of blister formation is not known. Since one of the main differences between local and systemic application is a possible contribution of the adaptive immune system, we asked whether T and B cells contribute to the delay in disease onset. We followed the hypothesis that after systemic application, the concentration of COL7-specific rabbit IgG at the dermal-epidermal junction may be too low for reaching the threshold for induction of a dermal-epidermal separation and that newly formed mouse IgG would support induction of skin lesions by binding to the rabbit IgG at the dermal-epidermal junction.

Our results demonstrate a strong T cell proliferation, an increased expression of the Th2 cytokine IL4 and the formation of germinal centers in draining lymph nodes within 3–6 days after subcutaneous injection of rabbit anti-COL7 IgG. To exclude any effects from mouse-derived IgG, B cell-deficient JHT/B6 mice were used for induction of EBA. Interestingly, a milder disease severity in B cell-deficient mice was found. This indicates that mouse IgG contributes to the pathogenesis but is not crucial. Further studies are needed to clarify this disease-supporting role of B cells in the passively induced EBA model.

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Calcineurin-dependent NFAT signaling is critical for anergy induction in regulatory T cells generated by human tolerogenic dendritic cells

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Our previous studies indicated that human IL-10 modulated tolerogenic dendritic cells (IL-10DC) are capable to induce anergic regulatory T cells (iTregs) that potently inhibit activated effector T-cell responses. Since NFATc-dependent transcriptional processes can contribute to T-cell tolerance, in this study we analyzed the function of T cell-related NFATc1, c2 and c3 molecules for the induction and function of human iTregs. For this purpose, we initially investigated the nuclear expression and shuffling of NFAT proteins in iTregs as compared to effector T cells (Teffs, primed with fully mature DC) after induction and restimulation of the T-cell populations. In contrast to Teffs, NFATc1 and NFATc2 were strongly translocated from the nucleus to the cytosol in activated iTregs in restimulation experiments, demonstrating an abrogated activation of these molecules in stimulated iTregs. However, after induction of iTregs we found a strongly enhanced nuclear expression of NFATc1 as compared to Teffs, suggesting an important role of NFATc1 for the priming of iTregs. The cytosolic/nuclear expression of NFATc3 did not show any significant differences between Teffs and iTregs. The activation of NFAT-related signaling is mainly regulated by the phosphatase calcineurin that de-phosphorylated NFAT molecules, thereby initiating the nuclear transport and induction of NFAT-mediated transcriptional processes. Therefore, we further investigated the effect of the calcineurin inhibitor Cyclosporin A (CsA) on induction and maintenance of the anergic state and regulatory function of iTregs. The experiments revealed that calcineurin inhibition by CsA during initial T-cell priming, but not during maintenance of the anergic iTreg phenotype, abolished the induction of the anergic state demonstrated by vigorous proliferation and enhanced IL-2 production. The impaired anergic phenotype of iTregs was associated with a rather naive T-cell phenotype (significantly decreased expression of CD45RO; ICOS, PD-1 and CTLA-4). Control experiments excluded an effect of CsA on the phenotype and function of IL-10DC or on the viability of iTregs. In conclusion, we demonstrated that NFAT-dependent signaling is crucially involved in the induction of human anergic iTregs generated by tolerogenic DC and we thereby identified NFAT-regulated transcription as target for iTreg-mediated tolerance.

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Nanoparticular targeting of tolerance inducing macrophages, DC and myeloid suppressor cells – new strategies for therapeutic approaches in immune-mediated diseases

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In recent years, nanoparticles (NP) have attracted a considerable attention for their ability to function as a possible carrier for target-specific delivery of various drugs, genes, proteins, peptides, vaccines, and other biomolecules in humans without much toxicity in order to target the immune system. Herein, antigen-presenting cells such as dendritic cells (DC), myeloid derived suppressor cells (MDSC) or M1/M2 macrophages are an interesting population as they modulate T cell responses in a stimulatory or inhibitory way. Therefore, the targeting of those cell populations using NP is of great interest for new therapeutic approaches in immune mediated diseases.

Polysaccharide nanocapsules built from crosslinked hydroxyethyl starch (HES) or dextran NP are both biodegradable, show a high loading capacity, provide the possibility of a targeted release of the cargo and thus seem to be ideal candidates for immunotherapeutic intervention. The particles used in our study were all below a diameter of 300 nm. In *in vitro* experiments we could show that incubation with these NP is non-toxic for PBMC, MDSC, DC and macrophages. Furthermore, NP lacked unspecific uptake by human peripheral blood mononuclear cells (PBMC), as shown by flow cytometric analyses and further confirmed using confocal laser scanning microscopy (CLSM). Moreover, when groups of tri-mannose were covalently linked to the surface of the HES nanocapsules, uptake was almost exclusively by monocytic cell-populations such as DC and macrophages.

Analysis of DC, macrophages and MDSC phenotype and functional modulation is currently being performed using flow cytometry of surface-molecules, and measurement of cytokine and chemokine production; the cells' stimulatory vs suppressive capacity is tested using mixed lymphocyte reactions. The obtained results will be validated *in vivo* using models of cancer metastasis to lungs and liver.

To conclude, the development of engineered NP as drug delivery systems offers enormous potential for breaking immune tolerance to tumors that is mediated by (innate) stromal suppressor cells. Especially, the targeting of molecules such as siRNA small molecules to specific innate suppressor cells through mannose derivatization via biodegradable NP is an attractive emerging option for the treatment of immune-mediated diseases.

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Activation of kappa-opioid receptor signaling ameliorates ongoing inflammation in the skin and the gut

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Opioids are known as powerful drugs for pain treatment. Although their widespread use is impeded by severe central side effects, opioids can induce potent analgesia without adverse effects by binding to peripheral opioid receptors. In this context, kappa-opioid receptor agonists (KORA) are of special interest since they have been shown to exhibit anti-inflammatory properties and, in contrast to agonists of other opioid receptors, are not associated with visceral side effects. As kappa-opioid receptors (KOR) are expressed on keratinocytes as well as immune cells and are up-regulated upon activation, we investigated the role of the newly developed KORA WOL071-007, comprising a perhydroquinoxaline scaffold and belonging to the class of arylacetamide KORA, during the progression of skin inflammation. Therefore, BALB/c mice were topically treated with imiquimod for 8 consecutive days to induce a psoriasis-like skin inflammation. After onset of disease mice were injected with KORA, an equal amount of an anti-tumor necrosis factor-α (TNF-α) antibody or PBS. Notably, compared to controls, recipients of KORA showed a significant reduction in skin inflammation, which was similar to mice that received anti-TNF-α. This was paralleled by a down-regulated expression of IL-23 as well as decreased levels of pathogenic Th1 and Th17 cells in lesional skin and regional lymph nodes as evidenced by immunofluorescence staining or flow cytometry. Since signaling via opioid receptors is known to be implicated in itching we quantified the scratching behavior of mice as well as the IL-31 mRNA expression in imiquimod-treated skin. Interestingly, in contrast to anti-TNF-α, KORA markedly reduced the scratching frequencies and down-regulated the IL-31 expression indicating that KORA ameliorated both, itch and Th1- / Th17-mediated inflammation. Of note, this effect was clearly mediated by binding of KORA to KOR since blocking KOR by co-injecting the specific antagonist nor-BNI into imiquimod-treated mice abrogated the beneficial effects of KORA during disease progression. Next, we investigated whether KORA was also able to ameliorate ongoing inflammation in other organs than the skin. As KOR has been shown to be up-regulated during intestinal inflammation we induced colitis in C57BL/6 mice by adding dextrane sodium sulfate to the drinking water. Notably, KORA prevented mice from weight loss and moreover, significantly reduced epithelial damage, ulceration and immune cell infiltration into colonic tissue. To further characterize the relevance of KORA for the inflammatory process in the gut we quantified neutrophils in the lamina propria as well as in mesenteric lymph nodes and assessed the myeloperoxidase (MPO) activity as a marker for neutrophil accumulation. In accordance with the histology showing less severe intestinal inflammation in KORA-treated mice compared to PBS-treated controls, the neutrophil counts and the MPO activity were decreased in KORA-injected animals. Importantly, also in DSS-induced colitis the anti-inflammatory potential of KORA was mediated by binding to KOR as we could completely abrogate the effect by blocking the KOR/KORA interaction with nor-BNI. Together, our data demonstrate that KORA, by binding to KOR, is able to ameliorate ongoing inflammation in the skin as well as the gut and down-regulates the proliferation and activation of pathogenic effector cells. Additionally, in psoriatic mice KORA reduced itching, thus suggesting KORA as a promising novel compound for the treatment of inflammatory/itchy disorders.

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Targeting the production of TNF-alpha, IL-6, IL-1beta and IL-12/23 of slan(6-sulfoLacNAc) dendritic cells has differential effects on innate and adaptive immune functions

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TNF-alpha, IL-6, IL-1beta and IL-12/23 are critical mediators of inflammatory skin diseases and are targeted by specific antibodies or soluble receptors. Mature 6-sulfoLacNAc positive dendritic cells (slanDCs) function as inflammatory dermal dendritic cells in psoriasis and cutaneous lupus and are a particularly rich source of TNF-alpha, IL-6, IL-1beta and IL-12/23. These cytokines have multiple effects on slanDCs themselves and on other immune and non-immune cells. Here we asked for the role of autocrine produced proinflammatory cytokines on the innate and adaptive immune functions induced by slanDCs.

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Immature slanDCs were purified from blood of healthy donors and cultured in the presence of inhibitors of TNFalpha and or IL12/23p40, IL1-beta and IL-6 (each 10 g/ml) added to immature (0 h) or spontaneously matured slanDCs (6 h of culture). SlanDCs were stimulated with lipopolysaccharid (100 ng/ml) at 6 h of culture and cytokine production and programming of allogeneic nave CD4+ T cells were studied.
Blocking of IL-6, IL-1beta and/or IL-12/23 did not significantly modulate the high level production of TNFalpha by slanDCs. In contrast, blocking of TNF-alpha largely reduced slanDC mediated IL-12p70, IL-23, IL-6- and IL-1beta secretion as well as programming of Th1 cells. Thereby, providing clear evidence that slanDCs require autocrine production of TNF-alpha to become highly proinflammatory dermal DCs as in psoriasis.
Anti-IL-12/23 did not affect TNFalpha-, IL-6- or IL-1beta-secretion by slanDCs, but blunted programming of IFN-gamma producing Th1 T cells by slanDCs. Taken together our studies identify TNFalpha as an important regulator for the functional maturation of slanDCs as innate and adaptive Th1-programming effector cells, while anti-IL-12/IL-23 primarily inhibited the capacity of slanDC to direct adaptive Th1 responses. This study appears critical for our understanding of how different functions of inflammatory dermal DCs – innate versus adaptive – are modulated by anti-inflammatory treatment strategies with biologics.

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Visualization of autoantibodies and neutrophils *in vivo* identifies novel checkpoints in autoantibody-induced tissue injury

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Over the past decades, autoimmune diseases have become a major medical burden. Many autoimmune diseases are characterized by presence of autoantibodies, and in some, such as autoimmune bullous dermatoses (AIBD), a clear pathogenic role of autoantibodies has been demonstrated. Pathogenesis of the effector phase of antibody-mediated diseases has been characterized in depth, and key cellular and molecular requirements for antibody-mediated tissue injury have been identified; e.g. neutrophils, cytokines, complement and Fc gamma receptors. The interaction of these key players and their exact timely and spacial interaction have however remained largely unsolved. To investigate interactions of autoantibodies and neutrophils in the organ targeted by the respective autoantibodies, we aimed to establish their visualization. For this, we selected epidermolysis bullosa acquisita (EBA), an AIBD with autoantibodies to type VII collagen (C7), as the skin is easily accessible for visualization by imaging techniques. Next, anti-mouse C7 IgG was fluorescently labeled. DyLight488- or DyLight594-coupled anti-C7 fully retained their blister-inducing activities, both *in vitro* and *in vivo*. When i.v. injected into mice, fluorescently labeled anti-C7 IgG rapidly (within minutes) bound to the dermal-epidermal junction (DEJ) as determined by multiphoton microscopy. Unexpectedly, this binding was not evenly distributed along the DEJ. We rather observed a patchy distribution of anti-C7 IgG along the DEJ. This led to the hypothesis that autoantibody binding may be affected by certain triggers, e.g. mechanical irritation. Indeed, mechanical stress, induced by scratching the skin without causing any histological alterations, led to an increased binding of anti-C7 to the DEJ. Next, fluorescently labeled anti-C7 IgG were injected into mice expressing eGFP under the control of the LysM promotor, and the subsequent events were again visualized by multiphoton microscopy. We here observed a rapid extravasation of eGFP expressing neutrophils and monocytes into the skin. Interestingly, these effector cells did not co-localize with the tissue-bound anti-C7 IgG. Co-localization of anti-C7 IgG with eGFP expressing cells were detected in parallel to the observation of clinically evident blistering, which occurred 4 days after the start of the experiment. Overall, this defines novel checkpoints for autoantibody-induced tissue injury exemplified in EBA: (1) Immediate autoantibody binding to the target tissue influenced by mechanical trigger factors, (2) rapid neutrophil recruitment into the vicinity of the autoantibody deposits and (3) delayed neutrophil recruitment to the autoantibody deposits and subsequent autoantibody-induced tissue damage.

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Proliferating Langerhans cells lead to reduction of neutrophils in chronic psoriatic inflammation

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Psoriasis is a chronic autoinflammatory skin disease of unknown etiology. Although a role for macrophages and dendritic cells in driving the autoimmune cascade has been proposed, its nature remains elusive. Topical application of Aldara cream containing the Toll-like receptor 7 agonist Imiquimod can induce psoriasis in patients. In mice, Aldara triggers pathological changes similar to psoriatic plaques. Using this model to study the onset of disease, a role of pDCs and Langerin- dermal Dendritic Cells has been proposed. However, the chronic phase of psoriatic inflammation is poorly studied. Therefore, we developed a long-term psoriasis model by continuing application of Aldara after plaques are formed and established. Using a high-resolution flow cytometry phenotyping key developed in the lab we desected all myeloid cells present at different times of inflammation. The cell infiltrate changes from predominantly neutrophils, pDCs, monocytes and Langerin- dermal Dendritic Cells in the acute phase to Langerhans cells (LCs) and macrophages in the chronic phase of inflammation. With the help of BrdU and Ki67 assays, the main part of this increase of LCs was revealed to be due to local proliferation of the epidermal LCs. However, using Ccr2 competitive chimera mice we detected some CCR2-independent influx from the bone marrow to the epidermal LC population. We then used LangDTR mice to specifically deplete LCs during both phases of psoriatic inflammation. In the absence of LCs there was a trend detectable towards stronger inflammation in established psoriatic inflammation. The absence of LCs correlated especially with an increased influx of neutrophils. Our results thus suggest a regulatory role of LCs in the chronic phase of psoriatic inflammation.

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The AhR-agonist 4-n-nonylphenol exerts immunosuppressive features by primarily modulating dendritic cells

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The arylhydrocarbon receptor (AhR) is a widely expressed ligand activated transcription factor. Its main function is the detoxification of small weight molecular toxins. It was shown that ultraviolet radiation (UVR) can activate the AhR in a ligand-independent fashion. This activation appears to be involved in UVR-induced immunosupppression, since AhR knock-out mice turned out to be resistant to UVR-induced immunosuppression. In turn, activation of the AhR by the agonist 4-n-nonylphenol (NP) inhibited the induction of contact hypersensitivity (CHS) and induced regulatory T cells (Treg) in a similar fashion like UVR. To identify the target cell in NP-induced immunosuppression, NP-treated and TNBS-coupled bone marrow-derived dendritic cells (BMDC) were injected s.c. into nave mice. Recipients turned out to be unresponsive to sensitization against the contact allergen TNCB and generated regulatory T cells, as demonstrated by adoptive transfer experiments. This indicated that NP exerted immunosuppression by primarily affecting antigen-presenting cells. Since IL-2 is a critical cytokine for the homeostasis, function and differentiation of Treg in the periphery, secretion of IL-2 by BMDC was measured. The analysis of supernatants revealed that upon stimulation with NP IL-2 production was significantly upregulated, indicating that NP-stimulated BMDC might induce peripheral Treg via enhanced secretion of IL-2. To further elucidate the underlying mechanisms, BMDC were cultured for eight days in the presence or absence of NP. A toxic effect of NP was excluded by death assays and annexin V/7-AAD staining. FACS analysis of NP-stimulated BMDC revealed unchanged expression of major histocompatibility complex class II. However, the negative regulatory molecules B7-H3 and 4 were significantly upregulated by NP, whereas the expression of B7-H1 was not affected. Together, these data indicate that NP might induce Treg by primarily modulating antigen-presenting cells and shifting their phenotype from a stimulatory into a regulatory one.

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Interleukin-33 promotes proliferation of mouse mast cells through ST2/MyD88 and p38 MAPK-dependent pathway

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Background: Interleukin-33 (IL-33), a member of the IL-1 cytokine family, is emerging as a new regulator of immune responses and inflammatory diseases. IL-33 signals via a heterodimer composed of IL-1 receptor-related protein ST2 and IL-1 receptor accessory protein (IL-1RAcP). IL-33 has been shown to activate T helper 2 cells (Th2), mast cells and basophils to produce a variety of Th2 cytokines and mediate allergic-type immune responses. Although IL-33 and its associated receptor ST2 appear to be expressed in mast cells, the precise role of IL-33 in modulation of mast cell function has not been determined.
Method: For the present studies, we employed *in vitro* differentiated bone marrow derived mast cells (BMMCs) from C57BL/6 mice. BMMCs were treated for 24–96 h with different concentrations of IL-33 and then checked for cell proliferation, degranulation, cytotoxicity, cell survival and apoptosis.
Results: IL-33 induces the proliferation of peritoneal and skin mast cell *in vivo* as determined by Ki-67 expression. IL-33 also resulted in increased proliferation of BMMCs, as explored by WST assay. Cell cycle analysis further confirmed the result as showing increased G2 cell populations in flow cytometry after propidium iodide staining. We further instigate that IL-33 mediated mast cell proliferation is dependent on ST2 as well as MyD88 receptor and mediated through p38 MAPK-dependent pathway. IL-33 did not induce degranulation on mast cell, as measured by β-hexosaminidase release, Cytotoxicity was unaffected at different times (24–96 h), as determined by lactate dehydrogenase (LDH) release in cell culture supernatants. Similarly, no effect was obtained by IL-33 on cell viability, as assessed by staining with the fluorescent dye calcein-AM.
Conclusion: We report here a novel role of IL-33 as an inducer of mast cell proliferation through ST2/Myd88 and p38 MAPK-dependent pathway. The findings may open new perspectives for understanding the role of IL-33 in different allergic diseases associated with mast cells.

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Specific targeting of murine and human CD4+ CD25+ T cells using IL-2 functionalized nanocapsules *in vitro* and *in vivo*

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Use of bioengineered nanocapsules (NC) as a drug delivery system to specific cell types in immunotherapy is of increasing interest due to higher efficacy with fewer side effects compared to systemically applied drugs. Approaches for dendritic cell targeting already showed promising results, whereas T cell targeting is still challenging because of limited endocytic activities. In this study, we generated IL-2 functionalized hydroxyethyl-starch (HES) nanocapsules for specific T cell targeting which exhibited a capsule size of below 200 nm. IL-2 was linked to the capsule surface via a PEG-linker with a copper-free click reaction. The biological activity of IL-2 bound to the capsule surface (HES-IL-2) was determined by proliferation assays using IL-2-dependent murine CTLL-2 cells and primary human CD4+ CD25+ T cells. Compared to control NC HES-IL-2 NC induced a vigorous proliferation of murine and human T cell populations. Further experiments excluded significant amounts of unbound IL-2 in the capsule supernatants. In addition, HES-IL-2 NC were significantly taken up by activated human CD4 + CD25high T cells after 72 h compared with control NC. IL-2 receptor mediated specific uptake by T cells was further verified by blocking IL-2 internalization with anti-IL-2 receptor antibodies. Laser scanning microscopy displayed that HES-IL-2 NC are located in the cytoplasm instead of membrane association. Analyses of the viability of T cells excluded a toxic effect after NC uptake. Intriguingly, for the first time to our knowledge, we were capable to control the amounts of IL-2 linked to the capsule surface and to quantify the cytokine after chemical bounding to the NC. Functional analyses revealed that equipment of NC with different amounts of IL-2 resulted in differences in the NC uptake as well as in the induced T cell proliferation. In order to investigate the function of HES-IL-2 NC *in vivo*, we i.v. applied HES-IL-2 NC into C57BL/6 mice. Here, we observed that IL-2 functionalized NC were taken up by lymph node T cells, whereas antigen presenting cells did not show a relevant uptake of HES-IL-2 NC 24 h after NC injection. The copper-free click reaction applied in the present study allows using sensitive proteins like IL-2 as targeting molecules for nanocapsules and in addition, enables us to link different amounts of the cytokine to the surface. Thereby, we are able to control the extent of the induced T cell activation and to select the targeted T cell population because of the CD25 expression. In future studies, drugs like siRNA or small molecules will be incorporated into IL-2 functionalized capsules to test their effect on melanoma models.

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Immune complexes as 'adhesion molecules' for inflammatory slanDCs

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There are a number of pathologic conditions where immune complex (IC) deposition or autoantibodies against endothelium cause Fc receptor-dependent inflammatory lesions. When exemplarily examining tissue sections of allergic vasculitis and lupus nephritis we recognized an accumulation of 6-sulfo LacNAc dendritic cells (slanDCs), a subset of highly proinflammatory human DCs that has been implicated in conditions like psoriasis or rheumatoid arthritis. To investigate possible routes of recruitment of slanDCs we applied a perfusion assay-based approach coupled with time-lapse video microscopy and measured arrest functions of purified leukocyte subtypes on immobilized ICs. The flow conditions were adjusted to provide physiologically relevant surface shear stress of human venous capillaries (>0.5 dynes/cm^2). Under these conditions we observed a pronounced recruitment of FcγRIII (CD16) positive slanDCs and NK cells to immobilized ICs while plasmacytoid DCs, CD1c+ DCs or T cells completely failed to adhere. When using immobilized human immunoglobulin subtypes instead of ICs we recognized a pronounced recruitment of slanDCs to human IgG3 but no other subtypes. However, enhanced recruitment of slanDCs to human IgG1 and IgG4 could also be observed when these subtypes were coincubated with an F(ab')2-specific

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crosslinking antibody, showing that the recruitment is not completely restricted to human IgG3. Investigating the receptor specificity of the recruitment of slanDCs by using specific blocking mAbs clearly showed that both the IC-mediated and the human IgG3-mediated attachment of slanDCs is largely dependent on CD16 while FcγRII (CD32) which is expressed by other DC subtypes plays, if at all, only a minor role. To extend our findings we then set up perfusion experiments over monolayers of dermal microvascular endothelial cells. Here again, we found a strictly CD16-dependent strongly enhanced recruitment of slanDCs to endothelial cells that were preincubated with an endothelial cell-specific antibody. Collectively, our findings demonstrate that the presence ICs in the vascular bed or anti-endothelial cell antibodies can overcome the need for classical adhesion molecules for the recruitment of circulating slanDCs and NK cells. This mode of recruitment may be especially important in IC-mediated tissue inflammation.

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Specific inhibition of p38-MAPK signaling suppresses experimental arthritis and inflammation induced hypoxia

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The p38-mitogen-activated protein kinases (MAPK) signaling pathway is critically involved in organ specific autoimmune diseases such as rheumatoid arthritis (RA)and can be therapeutically targeted by small molecule inhibitors. We have recently shown that hypoxia is critically involved in inflammatory immune responses such as experimental contact hypersensitivity reactions and arthritis. Importantly, inflammation induced hypoxia can be determined non-invasively *in vivo* in ankles of mice with glucose-6-phosphat-isomerase (GPI) induced arthritis already during the early onset of arthritic joint disease by using our well established hypoxia positron emission tomography (PET) tracer [18F]-Fluoromisonidazole ([18F]FMISO). The aim of our study was to elucidate whether the newly developed specific small molecule p38-MAPK inhibitor ML3595 is applicable to cure experimental GPI-induced arthritis and whether the treatment response can be monitored non invasively *in vivo* by detection of inflammation induced hypoxia using [18F]FMISO-PET imaging.

We induced experimental GPI-arthritis in nave BALB/c mice by injection of GPI antibody containing serum. Control mice were injected with serum derived from healthy C57BL/6 mice. We applied the p38-MAPK inhibitor ML3595 (30 mg/kg mouse) or sham-treatment (PBS) per os once daily starting three days prior to arthritis induction for ten days. The course of arthritic ankle swelling in the two experimental groups was measured once daily. [18F]FMISO-PET investigations were performed on days 3 and 6 after onset of GPI-induced arthritis induction in order to monitor the therapeutic potential of ML3595 *in vivo*. In addition, we performed histological analysis of arthritic ankles of ML3595- or sham-treated littermates (H&E staining).

ML3595 p38-MAPK inhibitor treatment yielded a reduced ankle swellings starting at day 1 after onset of GPI-arthritis. The maximum ankle swelling in both experimental groups was determined at day 6 after arthritis induction. At day 6 and day 8 after onset of GPI-induced arthritis the ankle swelling in ML3595 treated mice was 11–13% reduced when compared to the ankles of sham treated mice (day 6: ML3595: 3.30.4 mm; sham: 3.70.1 mm). [18F]FMISO-PET imaging of inflammation induced hypoxia in arthritic ankles on day 3 after onset of disease yielded no difference in tracer uptake between the two experimental groups (ML3595: 2.330.2%ID/cc, PBS: 2.280.2%ID/cc). On day six – in line with the reduced ankle swelling – the [18F]FMISO uptake in arthritic ankles of ML3595 treated mice was 15% reduced compared to the uptake in ankles of sham treated experimental mice (day 6: ML3595: 2.340.4%ID/cc, PBS: 2.750.2%ID/cc). Interestingly, histological analysis of arthritic ankles of ML3595-treated mice revealed despite the only 11-13% reduced ankle swelling a reduced pannus formation and joint destruction.

Treatment with the p38-MAPK inhibitor ML3595 revealed a reduced ankle swelling and joint destruction in mice with GPI-induced experimental RA highlighting the impact of p38-MAPK signaling in autoimmune diseases such as psoriasis arthritis. Finally, non-invasive *in vivo* [18F]FMISO-PET imaging of hypoxia seems to be a suitable tool for therapy monitoring, not during early stages but during advanced stages of experimental RA.

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Functions of MyD88 signaling in a mouse model of atopic dermatitis

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Myeloid Differentiation Primary Response Gene (MyD) 88 is a key molecule for pathogen recognition and the induction of inflammatory defense reactions by receptors of the innate immune system. MyD88-dependent receptors can also sense sterile danger signals in non-infectious inflammatory responses. Atopic dermatitis (AD) is a chronic allergic skin disease, possibly caused by skin barrier damage which leads to enhanced invasion of environmental antigens into the skin. The clearance of skin infections is also diminished in AD, which is attributed to a disturbed immune defense. Especially Staphylococcal Enterotoxin B (SEB) is involved in progression of the disease.

Naive MyD88-deficient mice exhibited elevated immunoglobulin E (IgE) levels, possibly pointing to enhanced allergy symptoms. However, in an Ova-dependent AD mouse model, MyD88-dependent signaling pathways were necessary for the induction of allergic inflammatory reactions. These included epidermal thickening due to keratinocyte hyperproliferation, Langerhans cell emigration from the epidermis, dermal influx of macrophages, cytokine production of antigen-specific lymph node T cells and the production of antibodies. MyD88-dependent signaling pathways were also involved in AD progression through co-administration of Ova+SEB.

The function of MyD88 in keratinocytes was analysed by Cre-loxP-mediated cell type-specific re-expression of MyD88 in MyD88-deficient mice. MyD88-induced signal transduction in keratinocytes was not sufficient to induce allergen-dependent epidermal thickening in the AD model. However it did promote Langerhans cell migration and reduced T cell infiltration into the dermis. In primary murine keratinocytes MyD88 was necessary for the production of inflammatory mediators which can promote Langerhans cell migration, such as IL-1alpha and GM-CSF. In contrast, the cytokine TSLP was produced in much larger amounts by MyD88-deficient cells compared to MyD88-proficient cells. TSLP might be involved in the diminished Langerhans cell migration and enhanced T cell recruitment seen in MyD88- deficient mice. Thus, MyD88-dependent signaling pathways are required for the interaction between keratinocytes and other cell types during the induction and course of AD. Additional studies may focus on the function of TSLP, as well as the role of MyD88 in keratinocytes during autoimmune reactions.

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Consecutive actions of adaptin 2 and casein kinase II regulate the intracellular trafficking of the dendritic cell antigen receptor DEC205 to and from antigen presenting compartments

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The dendritic cell (Dc) antigen receptor DEC205 mediates uptake of antigens into MHC-class II loading compartments in DCs. Its intracellular routing is guided by a short 31 amino acid intracellular domain (Dec tail). This domain harbours a putative targeting sequence (single letter code: EDExxL), which is thought to route lipoprotein receptors to lysosomes, as well as a sequence suitable for phosphorylation by casein kinase II (CkII). For our experiments we generated fusion receptors containing the extracellular human IgG-binding domain of the human FcγIII receptor (CD16) and the wild type intracellular domain (WT) of DEC205 (WT-DEC:CD16). This enables us to use FITC labeled human IgG (hIgG) as artificial ligand to trace the chimeric receptors. Site directed mutagenesis was applied to delete either the EDE sequence (AAA-DEC:CD16) or the CkII site (#Ck-DEC:CD16) in the DEC tail. The antigen presenting cell line DCEK was then stably transfected with the different fusion receptors and in pulse-chase experiments we monitored the intracellular routing of human IgG and its presentation to T cells. When analyzing the distribution of the DEC:CD16 receptors in steady state, i.e. without incubation with hIgG, we found that WT-DEC:CD16 receptors were present on the cell surface and in vesicles within the cells. AAA-DEC:CD16 receptors remained mostly close to the cell surface, whereas #Ck-DEC:CD16 was found close to the nucleus in golgi-like structures. When we analyzed the endocytosis of hIgG in different cell lines we found that WT-DEC:CD16 cells took up the ligand completely within 30 min, AAA-DEC:CD16 endocytosed only 10% of surface bound hIgG and #Ck-DEC:CD16 did not endocytose at all. Analysis of the intracellular transport of hIgG showed that in WT-DEC:CD16 hIgG moved to late endosomes (LE). In contrast, AAA-DEC:CD16 molecules remained mostly on the surface and only partly entered the early endosome (EE). We next tested the different cell lines for recycling of the DEC fusion receptors and found that WT- and AAA-DEC:CD16 recycled back to the surface, while #Ck-DEC:CD16 did not. Finally we revealed colocalisation of adaptin-2 with WT-DEC:CD16 but not with AAA-DEC:CD16 and colocalisation of #CkII-DEC:CD16 with the golgi marker TGN38. These results could further be supported by co-immunoprecipitation of the respective proteins. Thus, these data indicate that DEC205 interacts with adaptin 2 via its EDExxL motive, facilitating transport beyond EE. Subsequently the phosphorylation by CkII drives the recycling of DEC205 back to the cell surface. This shuttling makes DEC205 a highly efficient endocytic receptor for putative antigens.

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Breakdown of extracellular ATP by CD39+ Treg to adenosine induces migration of denditic cells via their adenosine A2A receptor

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We have shown previously shown that dendritic cells (DC) communicate with CD4+ CD25+ Foxp3+ regulatory T cells (Treg) via gap junctions during contact hypersensitivity reactions. To elucidate the mechanisms of these DC-Treg interactions, we set up *in vitro* cocultures of isolated cell populations. After 1 h we observed cluster formation between DC and Treg, but not among DC and conventional CD4+ T cells. The formation of aggregates between Treg and DC was preceded by enhanced migration of DC, which literally 'collected' Treg in culture vessels for the formation of large DC-Treg clusters. The migration of DC was dependent on adenosine produced by Treg, because Treg from CD39 KO mice, which lack the ectonucleotidase CD39 and are not able to convert ATP into adenosine, failed to initiate migration of DC and DC-Treg aggregation. Thus, we conclude that the production of adenosine by Treg is essential for guiding DC/Treg aggregation. To identify the responsible adenosine receptor(s) on DC we applied adenosine receptor antagonist's specific for the four different adenosine receptors A1, A2A, A2B and A3 to the DC-Treg cocultures. Here we clearly determined that only the adenosine A2A receptor antagonist blocked the adenosine induced motility of the DC and the formation of clusters with Treg. Thus, the adenosine effect was mediated by A2A receptors. As the A2A receptor enhances intracellular cAMP levels, we further dissected the putative downstream intracellular signaling events and found that cAMP drives EPAC1-mediated remodeling of the actin cytoskeleton, which is facilitated by redistribution of the actin remodeling proteins cofilin and CDC42 within the DC. This signalling cascade ultimately translates the chemotactic activity of Treg-derived adenosine into movement of DC. In summary, ATP is released in the skin during inflammation and allergic reactions and our data indicate that tissue resident Treg can degrade ATP into adenosine, which then mobilizes and attracts immature DC for their suppressive action. This may be an important pathway to prevent excessive immune reactions in the skin.

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Enhanced contact hypersensitivity reactions in CD73 deficient mice: role of migratory skin dendritic cells

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CD73, an ecto-5′-nucleotidase, plays a critical role in the extracellular conversion of ATP to adenosine. This is one determining pathway, deciding whether the extracellular environment is proinflammatory (characterized by ATP) or anti-inflammatory (characterized by adenosine). There are examples demonstrating that inactivating or inhibiting CD73 can cause exacerbated local and systemic inflammation in rodent models, but the details of the mechanisms are not known yet. To gain a better understanding of the role of CD73 during contact hypersensitivity reactions, we analyzed the immune reaction of CD73 deficient (CD37KO) and wild type mice during experimental CHS reactions. To induce CHS reactions, we sensitized groups of mice with 1% TNCB at the abdomen. After five days we determined the ear thickness and challenged the mice with 10 l 0.5% TNCB at the right ear. After additional 24 h we measured the ear thickness of the challenged ears. Here we recorded an approx. 3-times higher ear swelling reaction in CD73KO mice as compared to controls. Immunohistology of ears from CD73KO mice revealed an increased infiltration of T-lymphocytes. To reveal the underlying mechanism for the enhanced CHS reaction in CD73KO mice, we isolated lymphocytes from draining lymph nodes 72 h after sensitization and determined IFN- production by CD8 T cells by intracellular FACS. We observed increased numbers of CD8 lymphocytes in the CD73KO mice, which were producing about twice as much IFN-gamma as controls. As dendritic cells (DC) play a major role in priming immune responses against haptens we further investigated the regulation of DC functions in CD73KO mice versus controls. When analyzing the numbers of different skin DC subsets in unchallenged mice, no difference between controls and CD73KO were apparent. In contrast, upon challenge we found faster migration of dendritic cells from CD73KO mice out of the skin into the draining lymph nodes as compared to controls. Thus, these data indicate that absence of CD73 and its catalytic product adenosine, stimulates DC migration in CHS reactions, leading to accumulation of skin-derived LC/DC in respective lymph nodes. As a consequence enhanced activation of CD8 T cells and exacerbated CHS reactions will ensue.

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Characterization of dendritic cell subtypes in human melanoma and non-melanoma skin cancer

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In the skin, dendritic cells (DC) are the prime cells to induce immune responses against melanoma and non-melanoma skin cancer (NMSC), such as squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). Skin tumors are exceedingly common due to cumulative UV exposure and recurrent in immunsuppressed patients. Little is known about the occurrence, phenotypes and functional capabilities of tumor-infiltrating DC and their specific role in skin cancer.
The aim of our study is to to identify tumor-infiltrating DC subsets, their respective activation states and expression levels of C-type lectins by extensive FACS analyses. The tumor milieu will be investigated by analyses of tumor-infiltrating immune cells, such as immunsuppressive tumor-associated macrophages (TAM) and myeloid-derived suppressor cells (MDSC) as well as effector T and NK cells. By comparison of cutaneous tumors of melanoma, SCC and BCC we hope to learn more about the role of human skin DC in cancer. Preliminary data reveal the presence of different DC subtypes and activation levels in cutaneous metastases of melanoma, SCC and BCC. A substantial proportion of these DC express DEC-205 which would allow in situ targeting of tumor-infitrating DC. The TLR3 ligand poly(I:C) improves DEC-205 targeting in migratory skin DC, thus rendering it a promising adjuvant for immunotherapy. Moreover, infiltrates of T cells, NK cells and macrophages were obvious in all tumor entities. We are currently investigating interactions between these cells by immunofluorescence stainings of tumor sections.
This study will help to elucidate which DC subtype would be the optimal target for the clinical use of an 'antibody-targeting' immunotherapy.

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The role of langerin-positive skin dendritic cells in skin carcinogenesis

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Due to their localization skin dendritic cells (DC) are the first antigen presenting cells to get in contact with transformed keratinocytes forming squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). It is therefore critically important to understand how skin DC influence immune effector cells in skin prone to carcinogenesis and in fully developed tumors.
We used two different mouse skin cancer models: a cutaneous two-stage chemical carcinogenesis model forming SCC which we applied to LangerinDTR:EGFP mice and a SmoothenedM2 transgene model (Rosa26SmoM2YFP mice) further crossed to K5CreERT and LangerinDTR:EGFP mice to give rise to inducible BCC bearing mice which can be depleted of langerin-positive DC at various timepoints (SmoM2LangerinDTR mice).
So far experiments show that specific depletion of langerin-positive DC prior to the induction of SCC leads to a faster onset and higher number of tumors. Quantitative PCR analysis of skin shows a decrease in IL-12p40, TNF-a, IL-17 and IL-15 expression in the absence of langerin-positive DC two days after a single application of DMBA. Depleting langerin-positive DC in SmoM2LangerinDTR mice exhibit increased tumor load compared to non-depleted mice most probably due to enhanced proliferation of basal keratinocytes. Taken together, our first findings suggest that langerin-positive skin DC are required for early anti-tumor immune responses against non-melanoma skin cancer.

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OX40/OX40 ligand signaling enhances contact allergy by down-regulating the suppressive activity of Treg resulting in increased numbers of Th9 and mast cells

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The skin is constantly exposed to environmental factors resulting in the induction of immune responses. Previous work revealed that co-stimulatory molecules, like members of the TNF/TNF receptor family, are involved in the regulation of cutaneous immunity. The TNF receptor OX40 is expressed on T cells whereas its ligand (OX40L) is up-regulated on antigen presenting cells upon inflammation. To investigate the role of OX40/OX40L signaling during cutaneous immune responses we generated transgenic mice overexpressing OX40 in basal keratinocytes (K14-OX40 tg). Interestingly, after sensitization and challenge with the contact allergen oxazolone K14-OX40 tg mice developed a significantly increased ear swelling response compared to wildtype (wt) controls. As CD4+ CD25+ Foxp3+ regulatory T cells (Treg) are known to control contact allergy we analyzed the numbers and function of Treg in sensitized/challenged mice. Notably, OX40 ligation did not affect the numbers of Treg in lesional skin or regional lymph nodes but abrogated their suppressive activity by down-regulating the expression of CTLA-4, IL-10, Helios and TGF-β, all markers which have previously been associated with Treg function. To confirm that indeed the impaired suppressor function of Treg was responsible for the increased contact allergy in tg mice we adoptively transferred Treg from wt donors into sensitized K14-OX40 tg recipients resulting in the normalization of the ear swelling response to wt level. Since Treg inhibit the proliferation of effector cells during the progression of contact allergy and since OX40/OX40L signaling was able to block the suppressive activity of Treg we speculated that effector T cell numbers might be increased in tg compared to wt mice. Hence, we analyzed the effector cell populations in lesional skin and regional lymph nodes. As expected, we detected up-regulated numbers of proliferating Th2 cells expressing IL-4 and GATA3 in challenged ears from tg compared to wt mice. However, besides Th2 cells we observed enhanced levels of Th9 cells, which were characterized by the expression of IL-9 as well as the transcription factors STAT6, IRF4 and PU.1, in lesional skin and regional lymph nodes from K14-OX40 tg versus wt mice. Th9 cells have been shown to play a role during the progression of contact allergy since the secreted IL-9 is critically involved in the recruitment and activation of mast cells. Mast cells in turn were shown to act as regulators of contact allergy as the depletion of this cell population reduced the ear swelling response. Therefore, we next characterized the numbers and phenotype of mast cells in K14-OX40 tg and wt mice after elicitation of contact allergy. In fact, we detected markedly increased numbers of CD117+ mast cells expressing FcεRI, mast cell tryptase, MCP-1, IL-8 and histamine in tg mice compared to wt controls, thus suggesting that cutaneous OX40/OX40L signaling might have induced Th9 cells, which by secreting IL-9 recruited mast cells to lesional skin. To scrutinize this hypothesis we depleted IL-9 from sensitized/challenged tg mice by injecting a neutralizing antibody. The neutralization of IL-9 indeed resulted in reduced mast cell counts as well as a decreased contact allergy response in K14-OX40 tg mice. Together, these data indicate that in a contact allergy model cutaneous OX40/OX40L signaling down-regulated the suppressive activity of Treg resulting in the expansion of Th9 cells and the IL-9-mediated mast cell recruitment to lesional skin finally translating into an increased ear swelling response.

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GM-CSF and Glucocorticoids synergistically enhance retinoic acid production in monocytes with subsequent consequences for T cell fate

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During inflammation GM-CSF is produced in high amounts predominantly by T cells and circulating monocytes bind and respond to this pleiotropic cytokine. In parallel, Glucocorticoids (GC) are widely used to suppress inflammation clinically and we have shown that GC profoundly influence monocyte phenotype and function. Since monocytes function as effector cells and play an important role in regulating adaptive immunity we studied the influence of GM-CSF and GC on monocytes. We used murine bone marrow-derived monocytes and stimulated them simultaneously with GM-CSF and GC or with either stimulus alone for 48 h and analyzed phenotype and function of the resulting cells. Furthermore, we performed co-cultures of GC/GM-CSF-stimulated monocytes with autologous T cells and analyzed interaction of monocytes and T cells measuring cytokine production of T cells from the co-culture.
Interestingly, combined treatment of monocytes with GC and GM-CSF led to an increased cell viability and cell expansion compared to either GC or GM-CSF-treated monocytes. Phenotypically GC/GM-CSF monocytes were not distinguishable from monocytes that were either stimulated with GC or GM-CSF (CD11b, F4/80, Ly6C, CD121b, CD80) except for the T cell co-stimulatory molecule CD86 expression that was up-regulated in GC/GM-CSF-stimulated monocytes. We then tested for functional properties of the monocytes. Arginase activity was up-regulated by GM-CSF and not influenced by GC. On the opposite, nitric oxide (NO) production was up-regulated by GC treatment and not influenced by GM-CSF. GC/GM-CSF-treated monocytes, however, showed a synergistically increased retinoic acid (RA) production. Since RA e.g. is implicated in differentiation of regulatory T cells (Treg) we performed co-cultures of monocytes and nave T cells. Finally we measured Foxp3 as a signature molecule for Tregs and we also determined cytokine production of T cells from co-culture supernatants. Surprisingly, Foxp3 expression, however, was not increased by the RA-producing GC/GM-CSF monocytes in resulting T cells. Rather, pro-inflammatory cytokines IL-1 beta, TNF alpha and GM-CSF itself were enhanced in co-cultures of T cells with GC/GM-CSF-monocytes, while T helper cell-specific cytokines IL-4, IL-13 and IL-17 were not changed. Interestingly, IL-6 production was synergistically down-regulated and secretion of anti-inflammatory cytokine IL-10 was synergistically increased when GC/GM-CSF monocytes were co-cultured with nave T cells.
In conclusion, GC/GM-CSF-treated monocytes combine regulatory features by synergistically enhancing RA and IL-10 with adjuvant capabilities (IL-1 beta, TNF alpha, GM-CSF) that should be tested for their immunoregulatory capacity *in vivo*.

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Regulation of *Pseudomonas aeruginosa*-induced RNase 7 expression in keratinocytes

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Human skin protects itself by the release of antimicrobial proteins and peptides (AMP). One major skin-derived AMP expressed by keratinocytes is RNase 7. RNase 7 is constitutively expressed at a high level in keratinocytes and can be further induced by cytokines and bacteria such as *Pseudomonas aeruginosa*. We could recently show that treatment of primary keratinocytes with culture supernatants of *P. aeruginosa* led to a high induction of RNase 7. This, together with the known antimicrobial activity of RNase 7 against *P. aeruginosa*, indicates that RNase 7 may play an important role in cutaneous defense against *P. aeruginosa*. The signal transduction mechanisms mediating the *P. aeruginosa*-induced RNase 7 expression are still emerging and therefore we aimed to gain further insight into the underlying mechanisms. To this end primary keratinocytes were stimulated overnight with sterile-filtered culture supernatants of *P. aeruginosa* followed by analysis of RNase 7 expression using real-time PCR and ELISA. The *P. aeruginosa*-mediated induction of RNase 7 expression was reduced when culture supernatants of a flagellin-deficient *P. aeruginosa* mutant were used. This reduction of RNase 7 induction could be partly compensated by the addition of purified flagellin. These results indicate that flagellin is an essential component of *P. aeruginosa* to induce RNase 7. Since flagellin is known to signal via Toll-like receptor (TLR)-5 we used a TLR-5 blocking antibody to assess the role of TLR-5 in the *P. aeruginosa*-mediated RNase 7 induction in primary keratinocytes. Surprisingly, blocking TLR-5 did not led to a significant attenuation of the RNase 7 induction by *P. aeruginosa*, whereas the induction of the AMP human beta-defensin (hBD)-2 and psoriasin was significantly reduced by the TLR5-antibody. In concordance with these results, the induction of psoriasin, but not RNase 7, was reduced in keratinocytes treated with a MyD88-specific siRNA. Since MyD88 is an essential adaptor molecule in the TLR-5 pathway, these results further indicate that the TLR-5 pathway plays no essential role in mediating RNase 7 induction upon treatment with *P. aeruginosa* supernatants. This in turn suggests that the flagellin-dependence must be mediated via other pathways than the TLR-5 pathway. Future studies are necessary to identify the involved receptors and subsequent signal transduction pathways underlying the RNase 7 induction in keratinocytes stimulated by *P. aeruginosa*-released factors.

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TLR2-ligands promote chronic atopic dermatitis inflammation based on IL-4-mediated suppression of IL-10

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Atopic dermatitis (AD) is a T-cell mediated inflammatory skin disease with Th2 cells initiating acute flares. This inflamed skin is immediately colonised with *Staphylococcus aureus* providing potent TLR2 ligands. However, the impact of TLR2 ligands for the development of AD inflammation remains unclear. Using a model for acute AD with Th2 cells initiating cutaneous inflammation, we investigated consequences of TLR2 activation. We previously showed that Th2-cell mediated dermatitis is self-limiting and strictly depending on IL-4. We now demonstrate that activation of TLR2 converted this limited Th2-dermatitis into strongly enhanced cutaneous inflammation persisting for more than 14 days as seen in AD patients. This conversion was absent in TLR2−/− animals or when TLR2 was selectively absent in dendritic cells (DC). Analyzing underlying mechanisms we found that this conversion was based on the combinative sensing of both the innate TLR2 ligands and the adaptive Th2-cytokine IL 4. The concerted activation of TLR2 and IL-4R suppressed anti-inflammatory IL-10 *in vitro* and *in vivo* in immune cells residing in the skin such as in dendritic cells (DC). Using cells and mice from IL-10−/− and IL-4R−/− mice we identified that IL-10 is the key regulator of Th2-mediated dermatitis. The absence of IL-10 or the suppression of IL-10 by IL-4 in DC lead to dermatitis exacerbation and long-term dermatitis persistence. In summary our data demonstrate that innate TLR2 signals transform transient Th2-cell mediated inflammation into persistent dermatitis as seen in chronic human AD by IL-4 mediated suppression of IL-10. These data show for the first time how initial AD lesions transform into chronic inflammation and provide another rationale for targeting IL-4 in AD patients, a therapeutic approach currently under development.

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Fumaric acids inhibit the pro-inflammatory function of of 6-sulfo Lac NAc (slan) dendritic cells

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The fumaric acid dimethylfumarate (DMF) has immunomodulatory functions that allow long term control of widespread psoriasis. Underlying regulatory mechansims have been described at the level of cytokine expression or transcription factor activities in monocyte-derived dendritic cells (MoDCs), T cells, keratinocytes or endothelial cells.

None of these studies addressed the immunomodulatory function of fumaric esters at the level of human blood precursors of inflammatory dermal dendritic cells.

We recently described human 6-sulfo LacNAc-dendritic cells (slanDCs) as inflammatory dermal dendritic cells in psoriasis. In the skin slanDCs showed a strong expression of the key cytokines IL-23 and TNFalpha and *in vitro* they very efficiently programmed Th1- and Th17-T cells. Here we studied the effects of dimethyl fumarate (DMF) on the *in vitro* maturation and cytokine production of slanDCs. Immature slanDCs were purified with magnetic beads from blood and stimulated with the TLR7/8 ligand R848, thereby mimicking the *in vivo* TLR7/8-stimulation in the skin with RNA complexed to the anti-microbial peptide cathelicidin.

While DMF did not affect the viability of slanDCs it dose dependently prevented spontaneous maturation characterized by the upregulation of HLA-DR, the costimulatory molecules CD86 and CD80 as well as the DC-maturation marker CD83. Moreover, DMF-treated slanDCs showed an increase in cell surface expression of the inhibitory molecule PDL-1 but not of PDL-2. slanDCs are known as blood leukocytes with particularly high capacity to produce TNFalpha, IL-12 and IL-23. Notably, this high level production of pro-inflammatory cytokines was very efficiently blocked when cells were culture in the presence of titrated doses of DMF. In contrast to previous studies with monocyte-derived DCs we did not observe an increased production of the type II cytokine IL-10. Studies on intracellular signals as studied by western blotting provide evidence for a reduced phopsphorylation of NFkB p65 and no induction of HO-1 when slanDCs are cultured in the presence of DMF.

In conclusion, the remarkable clinical effects achieved with DMF in psoriasis may in part be attributed to its strong immunomodulatory effects on slan+ inflammatory dermal dendritic cells.

P175 (O36)

Stabilization of cAMP switches the cytokine profile for T cell programming of slanDCs from Th1 towards Th17

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slanDCs are a subset of highly proinflammatory myeloid dendritic cells which can be identified by the carbohydrate modification 6-sulfo LacNAc (slan). We recently identified slanDCs as inflammatory dermal dendritic cells in psoriasis. Characteristic for slanDCs is their spontaneous maturation, accompanied by gaining the potential for the production of high amounts of TNF-alpha and IL-12. During maturation, cAMP levels of slanDCs increase before they drop again at later stages of maturation. Here we investigated the effect of cAMP stabilization during maturation of slanDCs on their cytokine profile. To stably elevate intracellular cAMP levels we used two different substances: dibutyryl cAMP, which is a cell-permeable and stable analog of cAMP, and the phosphodiesterase 4 inhibitor roflumilast, which blocks the degradation of cAMP within the cell. Both substances inhibit the IL-12 secretion upon LPS stimulation by almost 90%. In contrast, the secretion of IL-23 and IL-1 beta is strongly enhanced by these two cAMP elevating agents with the same stimulation. IL-12 is known to program T cells towards a Th1 phenotype, whereas IL-23 and IL-1 beta are important for Th17 skewing. Thus, cAMP is important for the cytokine profile of slanDCs which in turn can influence the direction of T helper cell programming. Although the secretion of IL-10 is also upregulated by roflumilast and dibutyryl cAMP, we do not expect a skewing towards Th2 since the overall production of IL-10 by slanDCs is very low compared to e.g. monocytes. From our data we suggest a programming of nave T cells from Th1 to Th17 by stabilization of cAMP in slanDCs. Furthermore, these studies provide first evidence that therapeutic use of PDE4 inhibitors currently under study for the treatment of psoriasis and atopic dermatitis may have the potential to augment T cell mediated Th17 responses.

P176 (O15)

Insulin/IGF-1 signaling in myeloid cells coordinates skin inflammation

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Type 2 diabetes mellitus (DM) represents one of the major metabolic diseases associated with severe skin complications, including impaired wound healing and various inflammatory skin diseases, yet the mechanisms of action are not fully understood. Macrophages have been identified to play a pivotal role in the development of obesity-associated inflammation within the white adipose tissue, ultimately leading to Insulin resistance and type 2 diabetes. Recently, it was shown that direct action of Insulin on myeloid lineage cells is critical in this process. So far, it is unresolved, whether myeloid cells are a direct target of Insulin/IGF action in skin and whether Insulin/IGF signaling in myeloid cells plays a causal role in type 2 DM associated skin complications.

In this study we examined the role of cell autonomous Insulin/IGF signaling in myeloid cells in skin homeostasis and disease by generating mice that lack both the Insulin Receptor and the IGF-1 Receptor on myeloid cells (IR/IGF-1RMKO). IR/IGF-1RMKO mice were born and developed normally without any obvious skin phenotype. Unexpectedly, skin challenges that are characterized by an acute inflammatory response e.g. full thickness excision skin wounding, were similar in knockout mice when compared to controls. In contrast, in a model of chronic dermatitis (induced by topical application of sodium dodecyl sulphate (SDS)), IR/IGF-1RMKO mice were protected from skin inflammation, whereas control mice developed a severe skin inflammatory response characterized by acanthosis, hyperkeratosis and a mixed dermal inflammatory cell infiltrate. Gene expression analysis of skin lesions in control mice revealed a significant up-regulation of pro-inflammatory mediators in the epidermal and dermal compartment, when compared to mutant mice. In SDS-treated dermal tissue of mutant mice the number of macrophages was reduced but interestingly, also their activation phenotype was perturbed when compared to controls. Whereas, lesional dermis in control mice was dominated by a pro-inflammatory macrophage phenotype, macrophages in mutant mice rather showed the expression of immunosuppressive mediators. Gene expression analysis of macrophages stimulated *in vitro* with Insulin/IGF-1 corroborated our *in vivo* findings, indicating a critical pro-inflammatory action of Insulin/IGF-1 in dermal infiltrating myeloid cells. Myeloid cell-restricted IR/IGF-1R signaling thus appears dispensable in acute inflammatory processes, whereas it is critical to sustain chronic inflammation. Ongoing studies investigate the dynamics of IR/IGF-1R signaling in myeloid cells in acute versus chronic cutaneous inflammatory responses.

In conclusion, we provide evidence for a novel IR/IGF-1R-dependent pathway in myeloid cells that plays a critical role in skin inflammatory responses, and may add to the understanding of the molecular basis of type 2 diabetes associated skin complications.

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Janus kinase-2 inhibition ablates autoantibody-induced tissue injury

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Chronic inflammatory diseases have become a major medical burden. Despite improved diagnosis and treatment, morbidity and mortality remains high. Janus kinase-2 (JAK-2) is involved in downstream signalling of signal transducer and activator of transcription 3 (STAT3) and STAT5 and is responsible for activation of several inflammatory pathways. Inhibition of JAK-2 signalling is protective in several models of chronic inflammatory diseases, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) and its effects are currently evaluated in clinical trials. The role of Janus kinase signalling has not been evaluated in autoimmune bullous dermatoses (AIBD), in which autoantibodies to structural proteins of the skin lead to chronic (muco)-cutaneous blistering. Here, we describe the effect of CEP-33779, a selective, orally active, inhibitor of JAK-2 evaluated in experimental models of epidermolysis bullosa acquisita (EBA), an AIBD, caused by autoantibodies to type VII collagen (COL7). JAK-2 blockade dose-dependently inhibited immune-complex-induced reactive oxygen species (ROS) and elastase release from neutrophils. Furthermore, JAK-2 inhibition impaired blister-induction experimentally induced by incubation of human skin sections with anti-COL7 IgG and neutrophils. *In vivo*, EBA can be induced in mice by repetitive injections of anti-COL7 IgG. Prophylactic treatment of mice with CEP-33779 impaired induction of skin blistering in a dose dependent manner. This study demonstrates a key contribution of the JAK-2 signalling pathway in the pathogenesis of autoantibody-induced tissue injury in a prototypical organ-specific autoimmune disease. Our results also identified inhibition of JAK-2 as a potential therapeutic target, which is currently evaluated by treating mice with already established EBA with CEP-33779.

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Additive effect of non-digestible oligosaccharides on lactic acid bacteria induced secretion of anti-inflammatory IL-10 by human monocyte derived dendritic cells

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The combination of lactic acid bacteria (LAB) with neutral or acidic oligosaccharides similar to the composition in breast milk has been shown to harbor preventive effects towards immune-regulatory disorders. The aim of this study was to investigate the immune-modulatory potential of different bacterial strains in combination with non-digestible galacto- and fructo-oligosaccharides mimicking the natural distribution of oligosaccharides in human breast milk, assessing cytokine release by human monocyte-derived dendritic cells (MoDCs). Immature human MoDCs prepared from peripheral blood of healthy non-atopic volunteers were screened *in vitro* after stimulation with different LAB strains in the presence of specific neutral and acidic galacto- and fructo-oligosaccharide mixtures. Cytokine release by MoDCs was analyzed after 24 h in cell-free supernatants by ELISA.

Neutral and acidic oligosaccharide mixtures exert a significant additive effect on bacteria induced anti-inflammatory IL-10 secretion by MoDC, while no ability to increase pro-inflammatory IL-12p70 production was observed.

These results indicate anti-inflammatory and immune-modulatory properties of LAB in the presence of neutral and acidic non-digestible oligosaccharide mixtures *in vitro*. The tested combinations might represent a useful therapeutic strategy for immune regulatory disorders, such as skin diseases and could be considered as allergy preventing ingredients in food.

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Association of autoantibody specificities, HLA alleles, and site of clinical involvement in patients with mucous membrane pemphigoid

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Mucous membrane pemphigoid (MMP) is a subepidermal blistering autoimmune disorder with variable clinical involvement and antibodies directed against different proteins of the dermal-epidermal junction, including type XVII collagen (BP180), laminin 332, BP230, $\alpha6\beta4$-integrin, and type VII collagen. Serum autoantibodies are detectable in about 50% of MMP patients and are often present in low titers. Previously, several authors reported increased frequencies of HLA-DQB1*0301, HLA-DRB1*11 and HLA-DRB1*04 as well as decreased frequencies of DQB1*02 in MMP patients. To explore a possible correlation between clinical involvement, serum autoantibody specificities, and HLA-class-II alleles, we studied a well characterized cohort of MMP patients (n = 60; mean age 59 years) and controls (n = 45; mean age 61 years). A panel of 18 validated serological test systems used for characterization of autoantibody specificities was employed and HLA-DRB1 and -DQB1 were analyzed by allele-specific sequencing. In patients, HLA-DQB1*03:01 and DRB1*11 alleles were significantly more frequent than in controls, while HLA-DRB1*0301 was significantly lower in the patient group. Serum autoantibodies against the dermal-epidermal junction were detected in 68% (41/60) of patients; most showed antibodies against BP180 (42% of all patients, 61% of patients with detectable circulating autoantibodies) and in 36% (53% of patients with detectable circulating antibodies) against BP180NC16A, the immunodominant epitope of BP180. Because of its high specificity of about 91%, detection of antibodies against BP180NC16A with ELISA and/or biochip should be the first screening test in clinically suspected MMP. Combining these tests with detection of IgG autoantibodies by indirect immunofluorescence of salt-split human skin and monkey oesophagus, the clinical diagnosis of MMP could be confirmed in 75% of patients. MMP was associated with distinct HLA alleles and autoantibody specificities, but the identified serological and genetic patterns did not correlate neither with each other, nor with a specific pattern of clinical symptoms. Further studies are needed to identify genes outside the HLA locus associated with MMP and develop additional sensitive and specific assays for autoantibodies in this disease.

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P180

Investigation of autoimmune disease in humanized mice

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Our immunologic knowledge is mainly based on animal models, but species-specific differences between mice and men limit the prognostic value of these models for preclinical testing of novel biologicals. So-called humanized mice have been developed to overcome these limitations. We established such mouse models for effective testing of immunotherapeutic drugs *in vivo*. Immunodeficient NOD/Scid$\gamma c-/-$ mice were humanized with purified human CD34+ hematopoietic stem cells (HSC). This led to an engraftment of T, B, NK, dendritic cells and macrophages, which repopulate murine organs and initiate thymus and lymph node development. As a proof of concept, we evaluate the functionality of human immune cell by immunizing humanized mice with MOG protein. This resulted in a MS-like autoimmune disease including weakness of tail tone, paralyzed hind legs and neuroinflammation induced by human T and B cells. Immunologic characterization showed strong MOG-specific T cell proliferation in response to MOG-loaded DC and production of IL-17 and IFN-γ by human CD4+ Teff in peripheral blood of immunized mice. Furthermore, sick mice showed IL-6-mediated Treg unresponsiveness of T effector cells. In summary, our results show that humanized mice are beneficial for functional analyzes of immune reactions *in vivo* including allergy and autoimmunity. Furthermore, these models might be a promising tool for preclinical testing of novel immunotherapeutic drugs.

P181

Routes of internalization for Melan-A/TAT fusion peptides differ considerably between human dendritic cells and other non-phagocytic cell types

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Sufficient delivery of antigens into both MHC class I and II pathways combined with sustainable maturation of dendritic cells (DCs) is crucial for generation of effective immune responses. Multiple approaches for *ex vivo* antigen loading and improvement of immunogenicity have been described. We have recently established a single-step protocol consisting of a fusion peptide (cationic cell-penetrating HIV TAT domain and the melanoma antigen Melan-A) bound in complexes with the negatively charged Toll-like receptor 3 agonist Poly(IC). Since exact cellular uptake mechanisms of TAT-coupled antigens have been a matter of considerable debate and significantly depend on cell type, cargo and concentrations, we evaluated internalization routes into human immature DCs in comparison to non-phagocytic cell lines.

We found that Melan-A-TAT fusion peptide uptake by DCs is mainly energy-dependent, superior compared to Poly-Lysine-coupled Melan-A, and is significantly increased in DCs in contrast to Jurkat cells or HUVECs. Furthermore, we could track the uptake of the fusion peptide through early endosomes to lysosome compartments after 90 min by fluorescence micrcopy and immunoelectron microscopy. Specific endocytosis inhibitors revealed major internalization of the fusion peptide by DCs via Clathrin-mediated endocytosis, and, therefore, significantly differed from uptake by non-phagocytic HUVECs.

The data presented here gives new insights into the differential uptake of TAT-coupled peptides into professional antigen-presenting cells and other non-phagocytic cell types.

P182 (O10)

Do abnormal interactions between perifollicular mast cells and CD8+ T-cells contribute to the pathogenesis of alopecia areata?

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Mast cells (MCs) are crucial immunomodulators that play a role in murine hair growth control and in immune privilege (IP) maintenance. While an increase of MC number in alopecia areata (AA), a CD8+ T-cell dependent autoimmune disease of the hair follicle (HF), has been reported, their role and interaction with CD8+ T-cells in AA pathogenesis remains to be clarified. In this study, we compared by quantitative (immuno-)histomorphometry the number, degranulation, proliferation, expression of cytokines and co-stimulatory molecules of perifollicular MCs and characterized the cross-talk between MCs and CD8+ T-cells in defined perifollicular reference areas between lesional AA, non-lesional AA and healthy human scalp skin. Similar investigations were conducted in the well-established grafted C3H/HeJ mouse model of AA and in the newly-established humanized mouse model for AA.

These studies revealed a significant increase in the number, degranulation and proliferation of both, mature and immature perifollicular MCs in lesional AA skin compared to healthy controls and non-lesional AA skin, which was most prominent in subacute AA lesions. Perifollicular MCs of AA patients showed increased tryptase expression, but decreased TGFbeta1 immunoreactivity suggesting a MC switch from immune-inhibitory to pro-inflammatory activities. This hypothesis was supported by an up-regulated number of MCs expressing OX40L, CD30L, 4-1BBL and/or ICAM-1 while IL-10+ and PD-L1+ MCs were decreased, in AA lesional skin. Lesional AA-HFs also displayed significantly more peri- and intrafollicular- CD8+ T cells and physical MC/CD8+ T-cell contacts than healthy control skin, with MCs prominently expressing MHC class I and OX40L (and sometimes 4-1BBL or ICAM-1), in which MCs might present autoantigens to CD8+ T cells, provide co-stimulatory signals and/or promote activation, proliferation or survival of CD8+ T cells.

Abnormal MCs activities and interaction with CD8+ T-cells were also seen in the C3H/HeJ AA mouse model. Namely, the number of c-Kit+ MCs, mMCP6/tryptase release (degranulation) and contacts with CD8+ T-cells were increased in AA compared to sham-grafted control mice. Similar but less pronounced results were obtained in the humanized mouse model for AA, where the appearance of AA-like lesions in transplanted human scalp skin was characterized by an increase of MC number and MC-CD8+ T-cell contacts.

Taken together this suggests that perifollicular MCs in human and mouse AA skin are almost depleted of their immune-inhibitory properties and are skewed towards pro-inflammatory activities that facilitate cross-talk with CD8+ T-cells. This introduces the novel concept that pro-inflammatory MC activities and abnormal MC/CD8+ T-cell interactions contribute to trigger the HF IP-collapse in AA. Therefore, MCs may be a promising new target for managing AA and related T cell-dependent human autoimmune diseases.

P183

Efficient adoptive T cell therapy against melanoma in the absence of type I interferon system

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Background: Recombinant interferon (IFN)-alpha is in widespread clinical use for the adjuvant postsurgical treatment of patients with primary cutaneous melanoma at high risk for recurrence. However, the importance of endogenous type I IFN for immunotherapy is incompletely understood. Previous work in our lab showed that therapeutic activation of the type I IFN system with pI:C can expose immune cell-poor melanomas to innate immune surveillance. We hypothesized that in the absence of a functional type I IFN system the efficiency of immunotherapy against melanoma would be hampered.

METHODS: We treated cohorts of IFNAR1+/+ and IFNAR1−/− HGF-CDK4(R24C) mice bearing palpable primary melanomas in the skin with an adoptive T cell therapy protocol established in our lab. This treatment consists of preconditioning chemotherapy with cyclophosphamide, an adoptive transfer of nave IFNAR1+/+ or IFNAR1−/− gp100-specific pmel-1 TCRtg CD8+ T cells activated *in vivo* with Ad-gp100, and 3 adjuvant peritumoral injections of immunostimulatory nucleic acids CpG and pI:C. Additionally, we treated HGF-CDK4(R24C) melanomas transplanted in the skin of IFNAR1+/+ and IFNAR1−/− recipient mice. These model systems allowed us to evaluate the contribution of type I IFNs in the host.

Results: Adoptive T cell therapy caused regression, remission and relapse of primary IFNAR1+/+ and IFNAR1−/− HGF-CDK4(R24C) melanoma. The overall survival, pmel-1 T cell expansion and acquisition of effector functions were comparable in both cohorts of mice. Treatment of IFNAR1+/+ or IFNAR1−/− recipient mice bearing palpable transplanted HCmel3 melanomas in the skin with recapitulated the T cell expansion and acquisition of effector functions as well as tumor regression, remission and relapse observed in the primary melanoma model.

Conclusions: Taken together, our results unexpectedly revealed that a functional type I interferon system is not necessary for the expansion or acquisition of effector functions of adoptively transferred T cells and the efficacy of an adoptive cell transfer therapy against melanoma.

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Immune conditioning of the epithelial surface by skin microbiota enhances innate immune response towards pathogens

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Human skin is constantly exposed to a myriad of potential pathogens, while at the same time they allow harmless, non-pathogenic microorganisms to survive and colonize the tissue. Therefore, it seems that skin integrity is maintained by a kind of immune homeostasis in which the extent of skin immune response is controlled by active defence mechanisms and tolerogenic signals. We show that human keratinocytes in the epidermal layer of skin actively participate in the innate immune response towards pathogens by production of several cytokines and chemokines and antimicrobial peptides or proteins (AMPs) able to attract immune cells into the skin or directly kill the pathogens. We show that resident skin commensal bacteria create a protective environment by immune conditioning of epithelial surfaces. Interestingly, commensal bacteria are able to amplify the innate immune response of human keratinocytes to pathogens by activation of different signaling pathways acting in a synergistic way with pathogen induced pathways. Furthermore, we investigated how murine skin responds to *Staphylococcus aureus* skin colonization in a physiologic setting using an epicutaneous skin infection model. We show, that the efficiency of skin colonization correlated with the induction level of proinflammatory cytokines and AMPs. Our study suggests that skin barrier defects promote *S. aureus* skin colonization and that prolonged colonization is associated with profound cutaneous inflammation. Our data indicate that there is a crosstalk between immunomodulatory factors derived from pathogens and the host as well as between commensal, pathogen and host-derived peptides during bacterial infection of the skin. By this, keratinocytes as innate immune sensors are able to sense signals from the environment and initiate differential immune responses to harmless commensals or harmful pathogens, respectively. Current experiments address the protective effect of *Staphylococcus epidermidis* on skin infection and colonization *in vivo* in murine skin to provide deeper insight in skin immune responses to harmless commensals or harmful pathogens, respectively.

P185

Processing of laminin alpha chains generates peptides involved in wound healing and host defense

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Laminins play a fundamental role in basement membrane architecture and function in human skin. The C-terminal laminin G domain-like (LG) modules of laminin α chains are modified by proteolysis to generate LG1-3 and secreted LG4-5 tandem modules. In the present study, we provide evidence that skin-derived cells process and secrete biologically active peptides from the LG4-5 module of the laminin α3, α4 and α5 chain *in vitro* and *in vivo*. We show enhanced expression and processing of the LG4-5 module of laminin α3 in keratinocytes after infection and in chronic wounds in which the level of expression and further processing of the LG4-5 module correlated with the speed of wound healing. Furthermore, bacterial or host-derived proteases promote processing of laminin α3 LG4-5. On a functional level we show that LG4-5-derived peptides play a role in wound healing. Moreover, we demonstrate that LG4-derived peptides from the α3, α4 and α5 chains have broad antimicrobial activity and possess strong chemotactic activity to mononuclear cells. Thus, the data strongly suggest a novel multifunctional role for laminin LG4-5-derived peptides in human skin and its involvement in physiological processes and pathological conditions such as inflammation, chronic wounds and skin infection.

P186

Epidermal EGFR regulates cutaneous inflammation, antimicrobial defense and barrier function

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Cancer patients treated with Epidermal Growth Factor Receptor (EGFR) inhibitors (EGFRI) frequently develop acneiform skin toxicities, which are a strong predictor of a patient's treatment response. Here we show that the early inflammatory infiltrate of the skin rash induced by EGFRI is dominated by dendritic cells, macrophages, granulocytes, mast cells and T-cells. EGFRI induce the expression of cytokines and chemokines in epidermal keratinocytes, while the production of antimicrobial peptides and skin barrier genes is impaired. Correspondingly, EGFRI-treated keratinocytes facilitate lymphocyte

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recruitment, but show a significantly reduced cytotoxic activity against *Staphylococcus aureus*. Mice lacking epidermal EGFR show a similar phenotype, which is accompanied by chemokine-driven skin inflammation, hair follicle degeneration, decreased host defense and deficient skin barrier function as well as early lethality. Skin toxicities were not ameliorated in a Rag2, MyD88, and CCL2-deficient background and also not in mice lacking epidermal Langerhans cells. The skin phenotype was also not rescued in a hairless background demonstrating that skin inflammation is not induced by hair follicle degeneration. Our findings demonstrate that EGFR signaling in keratinocytes regulates key factors involved in skin inflammation, barrier function and innate host defense, thus providing important insights into the mechanisms underlying EGFRI-induced skin pathologies.

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Laser-assisted intradermal delivery of Xcl1-specific fusion vaccines induces potent anti-tumor response

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Dendritic cells (DCs) are highly efficient specialized antigen-presenting cells and have been regarded as promising targets in cancer immunotherapy. Cross-presentation of antigen by a subset of DCs, the CD8a-type DCs to CD8+ T cells is a fundamentally important mechanism in the defense against pathogens and tumors. In human and mice, CD8a-type DCs that are able to cross present uniquely express the chemokine receptor Xcr1 that binds the Xcl1 chemokine. We targeted antigens to cross-presenting CD8a-type DCs using Xcl1 inserted in dimeric vaccine molecules. Bivalent Xcl1 fusion vaccines bind specifically to and chemoattract CD8a-type DCs in a Xcr1 dependent manner.

With its easy accessibility and rich network of DCs, the skin appeals as a promising target site for vaccination. Using a laser microporation system, we specifically applied Xcl1 fusion vaccines into the murine dermis and targeted the cross-presenting CD8a-type dermal DCs. A single application of Xcl1 coupled to the modelantigen Ovalbumin (OVA) on the dermis of the mouse ear skin produced enhanced CD4 and CD8 T-cell responses, even in the absence of adjuvants. The first T-cell response was limited to the ear draining lymph node. This suggests that the application of antigens by laser microporation to the ear leads to a first localized immune response and thus limiting the possible amount of systemic side affects. However, such targeting of Xcl1-OVA also produced a striking enhancement of antibody responses. When the mice were challenged with B16 melanoma expressing OVA, treatment with the Xcl1-OVA could prevent development or mediate eradication of subcutaneous solid B16-OVA melanoma.

We conclude that targeting of dimeric fusion vaccine molecules to dermal CD8a-type DCs by use of Xcl1 represents a novel and promising method for inducing cytotoxic T cell reponses and a promising stategy to enhanve the efficiency of vaccines and immunotherapy.

P188

RANK-RANKL-activated Langerhans cells control MHC class I-mediated anti-viral immunity during Herpes simplex infection

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Cutaneous viral infections are controlled by the immune system and as RANK-RANKL interactions are involved in the regulation of immune responses we investigated whether this signaling pathway might modulate anti-viral immunity. Therefore, transgenic mice overexpressing RANKL in basal keratinocytes (K14 RANKL tg) were epicutaneously infected with Herpes simplex virus (HSV). Interestingly, K14-RANKL tg mice developed significantly smaller skin lesions compared to wildtype (wt) controls, which was accompanied by decreased virus replication in tg versus wt skin. Since innate effector cells such as NK cells or Gr-1+ cells have been implicated in anti-viral immunity we investigated the effect of RANKL overexpression on these cell subsets. However, similar numbers of NK cells and Gr1+ cells were detectable in infected skin and regional lymph nodes from wt and tg mice. Moreover, the depletion of either cell subset in tg mice prior to HSV challenge failed to increase the skin lesion size to wt level indicating a minor role of innate effector cells for the protection of tg mice from HSV infection. Next, we analyzed adaptive anti-viral immunity and identified 2-fold increased numbers of CD8+ T cells expressing cytotoxic markers in lesional skin and regional lymph nodes from tg mice compared to wt controls. Importantly, *in vitro* cytotoxicity assays revealed an HSV-specific cytolytic activity of these cells. To characterize the specific role of CD8+ T cells for anti-viral immunity we transferred CD8+ T cells from HSV-infected tg donors into wt recipients and infected recipient mice with HSV. Notably, transferred cells migrated to lesional skin and protected recipients from HSV infection. Next, we depleted CD8+ T cells from tg mice prior to HSV infection and could show that the lack of CD8+ T cells increased skin lesion size to wt level indicating that cutaneous RANK-RANKL signaling is critically involved in the regulation of MHC class I-mediated anti-viral immunity. After HSV challenge epidermal Langerhans cells (LC) are the primary dendritic cells (DC) getting into contact with the virus. However, LC are not the cells presenting HSV antigens to CD8+ T cells but transfer the antigen to CD103+ migratory dermal DC. In wt mice this process is hampered by the HSV-mediated induction of LC apoptosis. Since RANKL is known to increase cell viability we speculated that cutaneous RANKL overexpression might protect LC from HSV-induced apoptosis, which could result in increased antigen transport to regional lymph nodes and T cell priming. Indeed, the expression of apoptotic markers like caspase-3 or annexin V was absent in LC from infected tg skin. In support of our hypothesis we detected elevated numbers of CD103+ DC in regional lymph nodes of infected tg mice compared to wt controls. To elucidate the role of cutaneous RANKL signaling on LC function during HSV infection *in vivo* we depleted LC from tg skin. The ablation of LC increased skin lesion size to wt level and abrogated the expansion of HSV-specific CD8+ effector T cells. Next, we assessed whether the viability of LC and the induction of MHC class I-restricted anti-viral immunity could be improved in wt mice by intra-lesional injection of RANKL. Interestingly, soluble RANKL markedly decreased skin lesion size by increasing the viability of LC, enhancing the migration of CD103+ DC to regional lymph nodes, and up-regulating the priming of HSV-specific CD8+ effector T cells. Together, our data demonstrate that RANK-RANKL signaling is crucially involved in amplifying cutaneous MHC class I-mediated anti-viral immunity by protecting LC from apoptosis.

P189

Neutrophil inhibitory factor (NIF) from ancylostoma caninum inhibits neutrophil activation by autoantibodies against human type VII collagen (EBA autoantigen)

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Autoimmune subepidermal blistering dermatoses are prototypic autoantibody-mediated diseases. Autoantibodies are directed to structural proteins of the skin and cause tissue damage by activation of leukocytes at the dermal-epidermal junction. Necessary systemic immunosuppression therapy is often accompanied by severe side effects causing iatrogenic morbidity and mortality especially in elderly patients. Therefore, development of novel anti-inflammatory treatments with fewer adverse effects is a focus of current research. We here focused on epidermolysis bullosa acquisita (EBA), a subepidermal blistering disease, which is caused by autoantibodies to type VII collagen and mediated by neutrophil activation. For this purpose, we engineered a protein containing neutrophil inhibitory factor (NIF), derived from ancylostoma caninum (dog hookworm), which is characterized by its ability to inhibit neutrophil functions. Scalable expression of sufficient amounts was achieved by cloning NIF into a pMIB/V5-HisC expression vector for stable transfection of Sf21 insect cells. We found NIF to be secreted into the culture supernatant, from which it was purified by Strep-Tactin affinity chromatography. The biological activity of the NIF protein was evaluated in *in vitro* disease models that mimic the pathogenic events in EBA. As release of reactive oxygen species (ROS) is a precondition for blistering in EBA, we measured ROS release by neutrophil granulocytes from healthy volunteers stimulated with immobilized immune complexes and treated with different concentrations of NIF protein. Immune complexes consisted of recombinant fragments of human type VII collagen and chimeric human anti-human type VII collagen IgG1 autoantibodies. We found a dose-dependent inhibition of ROS production by NIF, with an EC50 of about 5 g/ml. On the other hand, toxicity of NIF for neutrophilic granulocytes proved to be low. We conclude that NIF is a good candidate for future selective inhibition of neutrophil functions in EBA and possibly other neutrophil-mediated subepidermal bullous dermatoses.

P190

Generation of the monoclonal antibody DD3 and its unique specificity for a subset of human CD16+ monocytes/dendritic cells

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Immune activation in the skin leads to recruitment and development of inflammatory dermal dendritic cells (DCs). Blood precursors of these DCs are not well characterized. Recently, we provided evidence that a subtype of CD16+ cells give rise to inflammatory dermal DCs. In psoriasis 6-sulfo LacNAc DCs (slanDCs) circulate in blood in an immature state, are found in and beneath the dermal vasculature and in the dermis locally express IL-23, TNFa and iNOS. SlanDCs share expression of CD16 (Fc*RIII) with a larger population of myeloid cells. A clear definition of these CD16+ myeloid cells is hindered by the lack of specific markers. To this end we set up a program to generate monoclonal antibodies (mAb) with specificity for CD16+ myeloid cells. BALB-c mice were repeatedly immunized with purified CD16+ cells giving rise to >1 × 10 monoclonal antibodies. The mAb DD3 turned out to be specific for a subset of CD16+, CD14low-negative blood cells. Double staining with anti-slan (clone M-DC8, DD1 or DD2) and the mAb DD3 revealed specificity for a CD16 expressing population overlapping but not identical with slanDCs. In fact, the two population overlap by 36%. For the phenotypic analysis by flow cytometry we could divide CD16+, CD14low-negative myeloid cells in three populations: (1) single mAb DD3+ cells, (2) cells double positive for DD3 and slan and (3) single slan+ cells. Population 1 expressed higher levels of monocytic markers (CD14, CD11b, CD33, CD45RO) compared to population 2 and 3. Next we performed multicolour cell surface staining combined with intracellular cytokine staining of PBMC stimulated with lipopolysaccharide. Population 1, 2, 3 as well as CD14+ monocytes, CD1c+ DCs and CD141+ DCs were studied. These studies revealed the highest IL-12p40/70 expression for slanDCs [population (2) and (3)], followed by the population stained positive with the new mAb DD3 [population (1)] but negative for slan. Taken together the mAb DD3 serves as a novel marker allowing for a more accurate functional and molecular definition of CD16+ myeloid cells in blood and in tissue. Our current phenotypic and functional data provide clear evidence for the presence of distinct subsets of CD16+ myeloid with slanDCs being apparently the most proinflammatory subtype.

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Tumour-derived monocytes change immune response in experimental leishmaniasis

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Monocytes play a pivotal role governing and regulating immune responses against pathogens. They phagocyte and kill parasites, produce cytokines and oxygen radicals to directly combat infections. In both animal models of cancer and clinical setting an accumulation of monocytes with immune suppressive function has been demonstrated. Thus, they suppress anti-tumour T cell responses and represent a newly detected important immune escape mechanism of tumours. Possibly, monocytes have a different functionality in a tumour environment and thus play a direct role in the susceptibility to infections of tumour patients.

We investigated whether tumour-derived cells not only down-regulates anti-tumour immune responses, but also immune responses directed against infectious agents. Therefore we transferred monocytes (CD11b-positive spleen cells) from melanoma-bearing mice (C57/Bl6) or control monocytes from nave mice into *Leishmania major* (*L. major*) infected C57/Bl6 mice. Subsequently, those C57/Bl6 mice which had received monocytes from tumour-bearing mice exhibited a higher parasite load than infected C57/Bl6 mice which had received control monocytes. Additionally, the reactivity of tumour-induced monocytes towards T cells was investigated. We found that CD11b positive spleen cells from tumour-bearing mice neither were able to induce allogeneic T cell stimulation nor to suppress T cell proliferation. To further characterise their immune function we performed *in vitro* studies. CD11b positive cells from tumour-bearing mice showed increased phagocytic capacity of *L. major* but were unable to mediate *L. major* killing.

These results imply that functionality of monocytes in a tumour environment is impaired. This might be a critical mechanism of immunosuppression in tumour patients hampering their immune response against to concomitant infections.

P192 (O22)

Role of HLA class II susceptibility alleles in the T cell-driven pathogenesis of pemphigus

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In Pemphigus, as in most autoimmune disorders, the strongest genetic susceptibility factor identified so far is the human leukocyte antigen (HLA). Pemphigus is considered as a model for an autoantibody mediated organ-specific autoimmune disease that is strongly associated with certain HLA class II alleles, especially HLA-DRB1*04:02 and HLA-DQB1*05:03. In contrast, in rheumatoid arthritis (RA), a systemic autoimmune disorder, the HLA-DRB1*04:01 allele is highly prevalent; HLA-DRB1*04:01 and 04:02 differ at an important position (DRß71) that is crucial for positioning of antigenic peptide in the HLA-DR pocket and that finally determines the activation of autoreactive CD4+ T cells by the HLA-DR-peptide complex. We are using the two humanized HLA-DRB1*04:02 and -DRB1*04:01-transgenic mouse models to investigate the impact of these

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genetic risk factors on the levels of cellular and humoral immune responses to human desmoglein 3 (Dsg3) the major autoantigen in pemphigus. A set of immunodominant, HLA-DRB1*04:02-binding, Dsg3 peptides which have been previously identified by autoreactive T cell clones in PV patients has been used for immunizing the HLA-transgenic mice. A control set included HLA-DRB1*04:02-non-binding Dsg3 peptides. Interestingly, while HLA-DRB1*04:02-transgenic mice that were immunized with the set of HLA-DRB1*04:02-binding peptides developed circulating Dsg3-reactive IgG antibodies as shown by indirect immunofluorescnce on human skin and human Dsg3-ELISA, those animals receiving the HLA-DRB1*04:02 non-binding Dsg3 peptides did not. These Dsg3-reactive antibodies were able to induce acantholysis upon injection into human skin biopsies and to disrupt cell adhesion of cultured epidermal keratinocytes. Moreover, HLA-DRB1*04:01-transgenic mice did not develop circulating Dsg3-specific IgG upon immunization with Dsg3 peptides at all. In draining popliteal and inguinal lymph nodes of HLA-DRB1*04:02-transgenic mice that were immunized with HLA-DRB1*04:02-binding Dsg3 peptides, both IL-4+ and IFN-γ+ T cells responded upon *in vitro* restimulation with the whole recombinant Dsg3 protein as determined by ELISpot assay. That was not the case neither in HLA-DRB1*04:02 transgenic mice receiving the HLA-DRB1*04:02 non-binding Dsg3 peptides nor in HLA-DRB1*04:01-transgenic animals. Our results clearly show that (i) CD4+ T cells restricted by the PV-associated HLA-DRB1*04:02 allele recognize a limited set of immunodominant Dsg3-peptides *in vivo*, (ii) activation of CD4+ T cells by HLA-DRB1*04:02-binding Dsg3 peptides induce the secretion of Dsg3-specific IgG antibodies and (iii) that mice transgenic for the RA-associated allele HLA-DRB1*04:01 do not produce any Dsg3-reactive IgG antibodies upon immunization with the different sets of Dsg3 peptides. In conclusion, HLA class II-transgenic mice provide a preclinical model to characterize the genetic susceptibility in pemphigus on CD4+ T cell and B cell levels. The identification of immunodominant CD4+ T cell epitopes of the autoantigen Dsg3 leading to the activation of autoreactive CD4+ T cells and finally resulting in the secretion of pathogenic Dsg3-specific IgG antibodies paves the road for new and innovative T cell-directed therapies in the future.

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Lithium-chloride induce interleukin 18 mRNA-expression and protein secretion in normal human epidermal keratinocytes

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Lithium is reported to be a trigger factor for psoriasis since many years. However, there are very few data regarding a possible mechanism of action of lithium salts used for treatment of depressive disorders and pathomechanisms involved in the generation of psoriasis lesions. Interleukin 18 is part of the inflammasome that is activated in psoriasis. Interestingly, IL-18 was found to be increased in metabolic serum and to play an important role in the pathogenesis of artherosclerosis both of which are known associated disorders in psoriasis.

To get a deeper insight into the mechanism by which lithium trigers psoriasis we investigated the effect on interleukin 18 (IL-18) mRNA-expression and protein secretion in normal human epidermal keratinocytes (NHEK) by lithium chloride.

NHEK were cultivated by standard proceedures and stimulated by flagellin, a known inducer of IL-18. Cells were treated with lithium chloride or culture medium. IL-18 mRNA expression was measured by real-time PCR and IL-18 protein in the culture supernatant by ELISA.

The results show that lithium chloride up-regulated IL-18 on both, the mRNA and the protein level in a dose-dependent manner in NHEK.

The data provide first evidence on how lithium salts can increase IL-18-driven pro-inflammatory processes in psoriasis.

P194 (O21)

Altered tumor necrosis factor receptor signaling in keratinocytes triggers interleukin-24-dependent psoriasis-like skin inflammation in mice

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Psoriasis is a common chronic inflammatory skin disease with a prevalence of about 2% in the Caucasian population. Tumor necrosis factor (TNF) plays an essential role in the pathogenesis of psoriasis, but its mechanism of action remains poorly understood. We have used several genetically modified mouse lines in addition to cultures of human and murine keratinocytes and expression analysis in human psoriatic skin to address the hierarchy of cytokine signaling in a mouse model of psoriatic skin disease and in human psoriasis. Here we report that the development of psoriasis-like skin inflammation in mice with epidermis-specific inhibition of the transcription factor NF-B was dependent on the presence of TNF receptor 1 (TNFR1) in epidermal keratinocytes. TNFR1 -dependent upregulation of interleukin-24 (IL-24) and activation of signal transducer and activator of transcription 3 (STAT3) signaling in keratinocytes were required for the inflammatory skin disease to develop. Deletion of the receptors for IL-19, IL-20 and IL-24/ IL-22 as well as of IL-22 revealed a pathogenic role for IL-24 in the development of psoriatic skin inflammation. IL-24 was strongly expressed in human psoriatic epidermis, and pharmacological inhibition of NF-B increased IL-24 expression in TNF-stimulated human primary keratinocytes, suggesting that this mechanism is relevant for human psoriasis. Moreover, IL- 24 expression was abolished in the skin of psoriatic individuals treated with TNF antagonists. Human epidermis reconstituted *in vitro* showed strong up regulation of cytokines and chemokines typically found in psoriatic skin upon stimulation with IL-24. Therefore, our results expand current views on psoriasis pathogenesis by revealing a new pathogenic mechanism that links TNFR1, NF-B, ERK, IL-24, IL-22R1, and STAT3 signaling to disease initiation.

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Contribution of T cells to autoantibody-induced tissue damage

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Epidermolysis bullosa acquisita (EBA) is an autoimmune blistering disease with autoimmunity against type VII collagen (COL7), an integral component of anchoring fibrils. In an antibody-transfer mouse model of EBA we investigated the contribution of T cells during immune complex-induced, neutrophil-dependent tissue injury. First, we demonstrated that T cell-deficient BALB/c nude mice are almost completely protected from EBA induction by transfer of anti-COL7 IgG. A similar observation was made when nude mice on the C57Bl/6 background where used, excluding strain-dependent effects. To exclude differences in neutrophil numbers and neutrophil function in wild type and nude mice, differential blood counts were compared, which were similar among the strains. I addition, immune-complex induced activation of bone-marrow derived neutrophils was similar among the strains. To exclude that the observed protection of nude mice from experimental EBA is due to an immune response to the injected rabbit IgG, we injected mouse anti-mouse COL7 IgG into ears of C57Bl/6 and C57Bl/6 nude mice. This led to the induction of subepidermal blistering in wild type, but not nude mice. Furthermore, the reconstitution of nude mice with T cells rescued the EBA phenotype after anti-COL7 IgG transfer, underscoring the importance of T cells for the modulation of neutrophil-dependent immune responses. In order to specify the responsible T cell-subclasses involved in tissue injury, we depleted different T cell subsets in mice. We identified NKT and gamma-delta T cells as the responsible subsets for susceptibility to experimental EBA. In summary, here we provide evidence that T cells modulate the immune complex-induced, neutrophil-dependent tissue injury.

P196 (O32)

Diverse T cell responses characterize the different manifestations of cutaneous graft-versus-host disease

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Graft-versus-host disease (GVHD) is the major clinical complication of allogeneic hematopoietic stem cell transplantation (HCT) and can present in an acute (aGVHD), a chronic lichenoid (clGVHD) and a chronic sclerotic form (csGVHD). It is unclear whether similar or different pathomechanisms lead to these distinct clinical presentations.

To address this issue, we collected lesional skin biopsies of patients suffering from aGVHD ($n = 25$), clGVHD ($n = 17$) or csGVHD ($n = 7$). We also obtained serial biopsies of non-lesional skin from HCT recipients at different time points prior and after HCT ($n = 14$). The cellular infiltrate was assessed by immunofluorescence stainings; interleukins and chemokines were measured by real-time RT-PCR. Cytokine profiles of stimulated T cells from collagenase-digested lesional skin biopsies were analyzed by intracellular flow cytometry.

While CD4+ and CD8+ T cells dominated the inflammatory infiltrate in both acute and chronic GVHD, the analysis of the quality of the T cell-mediated immune response revealed striking differences between the diverse forms.

In aGVHD lesions, there was a predominance of Th2 cytokines (IL-4, IL-5, IL-13) and Th2 chemokines (CCL17, CCL22). In accordance with these findings, the expression of TSLP, a keratinocyte-derived cytokine skewing the immune response towards a Th2 direction, was increased at day+20 post-HCT in non-lesional skin of patients who later developed aGVHD. Surprisingly, IL-22 but not IL-17 was also highly overexpressed in the acute but not in the chronic forms of cutaneous GVHD. In line with this finding, there was an increase of IL-22-producing CD4+ T cells infiltrating aGVHD skin.

In contrast to the situation in aGVHD, the immune response occurring in clGVHD was characterized by a mixed Th1/Th17 pattern. This was evidenced by a relative increase of Th1 chemokines (CCL5, CCR5, CXCL9, CXCL10), Th1 (IFN-gamma, IL-12/IL-23p40) and Th17 (IL-23p19) cytokines and a relative increase of IL-17- and IFN-gamma-single-producing CD8+ T cells. Chronic sclerotic GVHD also displayed a Th1 signature, showed an abundance of mast cells and exhibited a higher expression of the TRAIL-receptors TRAIL-R2/-R3/-R4 than clGVHD.

Our study provides new insights into the diverse pathomechanisms operative in the acute and chronic form of cutaneous GVHD. Our findings allow to more accurately distinguish aGVHD from clGVHD based on different cellular and molecular patterns. The analysis of TSLP expression in skin (before disease onset) could be helpful in identifying HCT recipients at risk for developing acute cutaneous GVHD.

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Novel Th-cell subsets within the GM-CSF producers in humans

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GM-CSF secreted by Th-cells plays an essential role in different murine models of chronic inflammatory diseases. However, the knowledge about GM-CSF production by human Th-cells is still limited. Here we demonstrate that more than 30% of human circulating Th-cells produced GM-CSF. Most of GM-CSF secreting Th-cells co-expressed IFNγ, indicating Th1-cells as major source of GM-CSF in humans. We observed that in Th1-cells the expression of GM-CSF was not influenced by IFNγ or IL-4 but was dependent on the action of calcineurin. In contrast to mice, only few Th-cells co-produced IL-17 and GM-CSF, likely due to the observed dose-dependent IL-17-decreasing while IFNγ and GM-CSF-increasing effect of the Th17-promoting cytokine TGFβ. Accordingly, we found two further Th-cell populations in the blood, whose ability to produce IL-17A, IFNγ, and GM-CSF was clearly differed from that of Th17- and Th1-cells. The first one of these Th17-Th1 transitional populations produced moderate levels of IL-17/GM-CSF/IFNγ, the second one is characterized by low IL-17 and high GM-CSF/IFNγ production. Importantly, 6% of blood Th-cells expressed GM-CSF in the absence of other Th-lineage cytokines, giving rise to the existence of a novel Th subtype in humans, ThGM-cells. In psoriatic lesions, the ThGM-cells and Th17-Th1 transitional populations were found even more frequently. All these populations might contribute to the pathology of psoriasis. In this common inflammatory skin disease, lesions contained elevated GM-CSF levels, which correlated with the expression of cytokines produced by antigen-presenting cells. Accordingly, GM-CSF mediated the upregulation of co-stimulatory molecules on myeloid cells.

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Contribution of the Aryl hydrocarbon Receptor Repressor (AHRR) to extrinsic skin aging *in vivo*

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The Aryl hydrocarbon Receptor (AhR) is a ligand activated transcription factor which is critical for detoxification of xenobiotics but is also important for the regulation of immunity. In addition to exogenous ligands like the polycyclic aromatic 2,3,7,8-Tetrachlordibenzodioxin (TCDD) the AhR is activated by endogenous ligands as 6-formylindolo[3,2-b]carbazole (FICZ). FICZ is a photoproduct generated from tryptophan in the skin. Thus, the AhR can be regarded as UV-sensor. AhR activity is regulated by feedback inhibition through the AhR-Repressor (AhRR) which is one of the target genes of the Ahr and therefore under direct control of the AhR. To date less is known about the physiologic function of the AhRR especially after exposure to UV-irradiation. To analyse the expression and function of the AhRR we generated AhRR-deficient mice by replacing the ahrr locus by an EGFP cassette, which allows monitoring AhRR expression. In the skin constitutive expression of the AhRR could be detected in epidermal Langerhans cells and dermal dendritic cells (DC), as well as in some keratinocytes and fibroblasts. AhRR expression was upregulated after AhR-activation *in vivo* and in primary keratinocytes and primary embryonic fibroblasts (MEF) *in vitro*. Interestingly, AhRR-deficient MEF exhibit reduced cell proliferation rates *in vitro*. First gene expression analyses revealed enhanced expression of cell cycle inhibitors, as p16INK4a, p19ARF and p21CIP in AhRR-deficient MEF

compared to wildtype MEF indicating a cell cycle arrest. The project aims to characterize the proliferative defect in AhRR-deficient MEF *in vitro* probably implying a senescent phenotype. Moreover, since several AhR ligands are associated with premature skin aging and the expression pattern of the AhRR in the skin might indicate an important role of the AhRR in skin immunity, we generated a model for both acute and chronic irradiation in order to analyse the impact of the AhRR after exposure to UVB.

P199

Systematic analysis of immunological changes in psoriasis patients undergoing treatment with ustekinumab

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Advancements in understanding the pathogenesis of psoriasis, including the role of T cells and cytokines, have been crucial to the development of biological therapies. These therapies strongly modulate the immune system by depletion of T cell effector cytokines (i.e. IL-17) or of priming cytokines required for the generation of pathogenic T helper cells (i.e. anti-p40), most importantly Th17 cells, which are the main players of autoimmune diseases. Ustekinumab (anti-p40) leads to significant improvement of psoriasis. However, the mechanism of action remains elusive since alterations in Th17 cell numbers and functionalities have been excluded recently. Interestingly, despite the strong perturbation of the human immune system with these therapies, patients suffer from few side effects. However, they entail the risk of detrimental infectious complications.

Therefore we set out to systematically investigate alterations of the human immune system in response to ustekinumab and other biological therapies as compared to healthy controls. Importantly, we assessed whether those therapies also affected the T helper cell response to opportunistic pathogens. This is expected to contribute to the identification of biomarkers for treatment responses and to the assessment of risk of infections in psoriasis patients with biological treatments.

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Mast cells possess several functional inflammasomes to respond to pathogen signaling with IL-1beta

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Introduction: Traditionally Mast cells (MCs) were seen mostly as effector cells of the adaptive immune system, since they can mediate anaphylactic reactions towards IgE crosslinking. However, MCs are emerging as key contributors to innate immune responses. Recently skin mast cells have been reported to be an important source of IL-1beta in patients with autoinflammatory conditions such as cryopyrin-associated-periodic-fever syndromes (CAPS). CAPS patients show IL-1beta-driven systemic inflammation together with non-histamine dependent urticarial rash, which are caused by activating mutations of the NLRP3 inflammasome, a multiprotein oligomer responsible for the initiation of inflammatory responses to pathogens.

Objectives: To determine if mast cells can produce and release IL-1β in response to pathogenic signals that target the inflammasomes NLRP3, NLRC4, or AIM2.

Methods: Peritoneal mast cells (PMCs) were obtained through lavage from adult (>8 weeks) C57BL/6 mice and WBB6F1 Kit+/+ mice, purified via CD117+ bead selection (>96% purity) and cultured for 7–14 days. 10^5 cells/well were primed with LPS (100 ng/ml) for 15 h. Then the PMCs were stimulated with 10 M Nigericin (NLRP3), 5 mM ATP (NLRP3), 100M R837 (NLRP3) for 45 min or for 4 h with 600 ng Flagellin (NLRC4) transfected with DOTAP or 200 ng polydAdT (AIM2) transfected with Lipofectamine. IL-1 beta production was measured in the supernatants by Elisa.

Results: PMCs produced significant amounts (mean SEM) of IL-1ß upon stimulation with Nigericin (467.41 pg/ml), ATP (152.88 pg/ml), R837 (21.2 pg/ml), Flagellin (245.44 pg/ml) and polydAdT (571.194 pg/ml) without stimuli the IL-1ß was (7.1 0.8 pg/ml) only.

Conclusion: We show for the first time that mouse mast cells incubated with inflammasome activators produce significant amounts of IL-1ß *ex vivo*. Inflammasome activation in MCs could play a critical role in other inflammatory skin conditions and innnate immunity responses to pathogens. Further research will analyze the inflammatory response of mast cells in urticaria and pharmacological inhibitors of inflammasome activation.

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Regulation of GM-CSF production by human T helper cells

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GM-CSF is a hematopoietic growth factor with pleiotropic functions. Previous studies on experimental autoimmune encephalomyelitis (EAE) have demonstrated an essential pathogenic role for T cell derived GM-CSF in autoimmunity. However, the role of GM-CSF in human inflammatory diseases has not been established yet nor has its regulation in human T helper cell subsets been addressed. In this study we report the existence of GM-CSF producing human T helper cells *in vivo* that lack co-expression of other lineage defining cytokines such as IFN-γ, IL-4 and IL-17 and their respective transcription factors T-bet, GATA-3 and ROR-γt. Although this suggests the existence of a novel T helper cell subset we could demonstrate that the classical Th1, Th2 and Th17 cell subsets could acquire GM-CSF production abilities. Therefore GM-CSF production was not restricted to a separate T cell subset but also a universal feature of other polarized human T helper cell subsets. To address its role in autoimmune pathogenesis, we isolated T helper cells from psoriasis plaques and healthy skin and compared their cytokine profiles. T cells from diseased skin had higher expression of IL-17 and IL-22, two cytokines reported in the pathogenesis of psoriasis, but lower levels of GM-CSF than T cells in healthy skin.

Our results suggest that GM-CSF cannot be considered a major driver of autoimmune tissue inflammation in humans in contrast to mice due to its expression by all T helper subsets and its reduced production in inflammatory skin tissue. The physiological role of memory T cell derived GM-CSF remains to be identified.

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Aged mice accumulate hyper-responsive autoreactive T cells and impaired hypo-responsive T cells characterized by specific phenotypes and ROS profiles as features of immunosenescence

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Aging of the immune system, termed immunosenescence, is characterized by a functional decline leading to an onward immunodeficiency. Since both host defence and regulatory mechanisms decline, this altogether supports infection, tumor and autoimmune disease with high prevalence in the elderly.

We here characterize distinct age-dependent T cell subpopulations showing a profile of dysfunctional and autoreactive T cells in peripheral lymphoid organs of young, adult and old mice (3–6, 9–12 and 18–24 months-old C57BL/6, $n > 5$). The majority of the CD4+ T cell subset showed an age-related gradual reduction of important costimulatory receptors, CD27 and CD28, and down-regulation of CD5. Only a minority of the CD4+ T cell subset showed a CD27−/low CD28−/low CD5low phenotype indicating homeostatic expansion, which typically occurs due to a reduced thymic T cell output with age. Also CD8+ T cells and CD4− CD8− double-negative (DN) T cells had this age-related CD27−/low CD28−/low CD5low phenotype suggesting these cells had an age-related increased reactivity. Importantly, these latter cells are characterized by an autoreactive TCR profile supporting autoimmunity while impairing regulation.

Recent reports indicate that imbalanced levels of reactive oxygen species (ROS) contribute critically to driving chronic inflammation and immunosenescence. Therefore, we used high-throughput eight-channel fluorescence FACS to further analyze the age-dependent CD27−/low CD28−/low CD5low T cells regarding their ability to generate ROS. As a result, CD27−/low CD28−/low CD5low T cells showed a markedly impaired ROS response to the mitochondrial electron chain complex inhibitor rotenone *ex vivo*, measuring decreased O_2- radicals and H_2O_2. Such decreased levels of ROS result in reduced rather than oxidized cell surface proteins thereby increasing the activity of this autoreactive T cell subset. In comparison phenotypically normal naive and antigen-experienced T cells from old mice showed higher ROS production, turning down their activatability. Besides, T cell subsets from old mice showed significantly elevated O_2- radical production and diminished H_2O_2 production especially in the CD8 T cell compartment.

In summary, our data reveal a substantial increase of pathogenic T cell subsets in old mice leading to an increased immune dysfunction. The decreased H2O2 production *in vitro* was paralleled *in vivo* by impaired extracellular ROS response against lipopolysaccharids (LPS). Since also the antigen specific proliferation induced by old bone marrow-derived APC was reduced, we hypothesize that lymphocytes and also bone marrow-derived cells acquire an age-dependently increasing hyporesponsiveness. Our data may contribute to clarify important aspects of an age-associated dysfunction of T cells in an aging immune system responsible for infection, tumor and autoimmune disease in the elderly.

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Glucocorticoids enhance anti-inflammatory and migratory capacities of murine monocyte

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Glucocorticoids are common used anti-inflammatory drugs. But detailed mechanisms of their mode of action are still unknown. We recently showed that glucocorticoids induce a specific monocyte phenotype with anti-inflammatory properties in humans. These glucocorticoid stimulated monocytes (GCsM) display increased chemotaxis, produce anti-inflammatory mediators like IL-10 and show higher capacity for phagocytosis of latex beads, bacteria and Leishmania (L.) major parasites.

In order to analyze the functional properties of GCsM in-vivo and their relevance for infections, we investigated whether there is a murine counterpart of the human GCsM.

We revealed that glucocorticoid treatment of murine monocytes results in a similar anti-inflammatory phenotype including functional features like low adhesiveness, but high migratory capacity to chemotactic factors like C5a and leishmania chemotactic factor *in-vitro*.

Using monocytes tagged with the near-infrared emitting dye DiR and *in-vivo* imaging, we were able to demonstrate that GCsM have a higher migratory capacity in different inflammatory model systems *in-vivo*. Genome wide gene expression analysis revealed 205 up-regulated and 190 down-regulated genes after stimulation with glucocorticoids and that expression of parts of the migratory machinery is affected by glucocorticoids. Increasing chemokinetic capabilities may caused by down-regulation of adhesion molecules and up-regulation of Myosin X. Latter is known for his intrafilopodial motility, functions in axonal path-findings and phagocytosis. These properties, together with down-regulation of integrins could provide GCsM a very fast membrane vesicle turnover into the filopodia, leading to f-actin intrusion facilitating fast migration.

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Reversible suppression of normal thymic output in patients with leukemic cutaneous T cell lymphoma

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Patients with leukemic cutaneous T cell lymphoma (L-CTCL) have a number of unexplained immune abnormalities, including widespread neutrophil activation, marked losses of circulating T cell diversity by spectratyping and compensatory proliferation of surviving T cells. We now report that patients with L-CTCL also have reduced circulating nave T cells. Although the number of nave T cells in healthy individuals decreases gradually with age, a significant number of nave T cells are present in the blood of older individuals and are thought to arise from both continued thymic output and from an increased lifespan of nave T cells in older individuals. The absolute numbers/ml of benign nave T cells (CD4+ CD45RA+ CD45RO−) was significantly decreased in patients with L-CTCL (systemic CTCL) as compared to healthy controls whereas the number of nave T cells in patients with MF (localized CTCL) did not differ from healthy controls. In healthy individuals and MF patients, numbers of nave T cells declined gradually with age, but nave T cells in patients with L-CTCL were markedly reduced regardless of age. Loss of nave T cells occurred uniformly in L-CTCL patients regardless of whether the total white count was low, normal or elevated. CD31, a marker of recent thymic emigrants, was decreased on the few remaining nave T cells in L-CTCL patients, suggesting ongoing decreased thymic output. Successful treatment of L-CTCL patients, regardless of modality, was associated with increased T cell receptor excision circles (TREC) and restoration of circulating nave T cells, most of which were CD31+ and thus represented recent thymic emigrants. Our results suggest thymic output of nave T cells is suppressed in L-CTCL, perhaps contributing to the immune deficits observed in these patients. However, this suppression is reversible and thymic output and nave T cell counts recover following successful therapy.

Infectious Diseases

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Human sweat – the natural environment of the antimicrobial peptide dermcidin

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Eccrine sweat glands in humans are distributed over the entire body and secret a fluid which is a rich source of functionally important cellular proteins and ions influencing skin physiology and function. One abundant protein in human eccrine sweat is Dermcidin (DCD), which gives rise to DCD-peptides

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with a broad spectrum of antimicrobial activity against pathogenic microorganisms (*Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis* and *Candida albicans*). Post-translational modifications modulate the activity of many eukaryotic proteins and it is yet unknown whether proteins in eccrine sweat exhibit post-translational modifications. As DCD peptides also possess potential acetylation and phosphorylation sites, we analyzed human sweat samples by mass spectrometry for existent modifications.

The physiological conditions in which DCD peptides are functioning are not completely elucidated yet. In this regard we could identify the organic acids and amino acids present in human sweat and thereby succeeded to compose a medium resembling human sweat. Using this medium we can mimic the physiological situation on human skin and analyze the activity of human skin-derived antimicrobial peptides under natural conditions. Furthermore, we performed proteome analysis of washing fluid from healthy human skin comprising proteins secreted into sweat and from keratinocytes. The analysis might help us to get more insights into the interplay of antimicrobial peptides and other proteins on human skin.

P206

Involvement of STAT1 signalling in Th17 and Th22 differentiation in patients with chronic mucocutaneous candidiasis

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Patients with chronic mucocutaneous candidiasis (CMC) carrying heterozygous gain-of-function (GOF) STAT1 mutations show a defect in IL-17 and IL-22 producing T cells pointing to an important role of STAT1 in the regulation of IL-17 and IL-22. However, mechanisms by which these STAT1 mutations impair IL-17/IL-22 immunity are not clarified so far. Aim of the study was to investigate the role of STAT1 in the regulation of IL-17 and IL-22 by analyzing T cell immunity in CMC patients with an overactive STAT1 protein on the one hand and STAT1 deficient mice on the other. Cytokine secretion dependent on STAT1 in PBMCs of CMC patients was examined using the STAT1 inhibitor fludarabine. PBMCs of CMC patients ($n = 4$) and healthy controls ($n = 4$) were stimulated with Candida albicans or aCD3/aCD28 in the presence or absence of fludarabine. Cytokine production was characterized by ELISA and flow cytometry. Moreover IL-22 expression in activated splenocytes from STAT1−/− mice was analyzed by ELISA and FACS. Cytokine analysis revealed that STAT1 inhibition by fludarabine improves the defect Th17 response in CMC patients mirrored by an IL17A and IL-22 induction after the stimulation with *Candida albicans* in the presence of fludarabine. Inversely to impaired IL-22 and IL-17 production in patients with STAT1 over-reactivity, STAT1−/− mice show a higher production of IL-22 and IL-17 in splenocytes compared to WT mice. These data indicate that a gain of STAT1 activity impairs IL-17/IL-22 responses. The defect Th17 response in CMC patients can be partially restored by the inhibition of STAT1 suggesting a possible therapeutic intervention in CMC patients. Murine data strongly support the role of STAT1 in the regulation of IL-17 and IL-22. In summary this work provides the basis for the development of new therapy strategies for the defect Th17 immunity in CMC patients with STAT1 overexpression.

P207 (O06)

Von Willebrand factor mediates the binding of *Staphylococcus aureus* to the endothelium upon physiological blood flow conditions

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Staphylococcus aureus is a frequent commensal bacterium colonizing skin or mucous membranes. However, entrance into and systemic dissemination through the body via the vascular system is often associated with severe diseases such as endocarditis or sepsis.

Adhesion to the vessel wall and internalisation into endothelial cells is a crucial step during pathogenesis and related to the virulence of the invading strain. *In vitro* microfluidics as well as *in vivo* intravital microscopy confirmed the cardinal involvement of von Willebrand factor (VWF) as initial binding partner for staphylococci floating in blood. Blood-flow dependent elongation of VWF and thus exposure of the multi-adhesive A1 domain was not only a requirement but confers remarkably high binding strength that allows the sequestration of large bacterial clumps to the intact endothelial surface. Applying a physiological shear stress of 10 dyn/cm, VWF-mediated binding resisted drag forces that steeply increased with the diameter of the attached bacterial cluster. Accordingly, biological relevant forces ranged between 8pN for a single bacterium with a diameter of 1 m towards 5000pN for bacterial clusters with diameters of 20 m. In comparison, fibronectin-mediated bacterial adhesion to the endothelium has been measured by others to resist drag forces of only 10 to 30pN. Significantly reduced binding of various mutant bacterial strains including *S. aureus* lacking wall teichoic acid, sortase A or the extracellular adhesion protein suggest a highly complementary interaction mechanism underlining its significance during bacterial spreading. Further data suggest the contribution of other host-derived molecules such as DNA or galectin-3. While extracellular DNA led to a twofold elevation of endothelium-bound bacteria, galectin-3 appears to attenuate the elongation of VWF and thereby the tethering of *S. aureus*.

Prevention of VWF adhesiveness due its proteolytic inactivation by ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) or due to the blockage of the A1 domain by heparin envisions new therapeutic strategies acting against systemic *S. aureus* infections.

P208

Human vitamin D-dendritic cells regulate T cell-mediated cutaneous host defense

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Vitamin D deficiency has been clinically linked to an increased risk for both autoimmune and infectious diseases in humans. Moreover, vitamin D-treated tolerogenic dendritic cells (vitamin D-DCs) are being explored as potential therapeutics in the context of autoimmunity given their ability to promote Treg and Th2 T cell responses. Yet, the effect of vitamin D on the ability of human DCs to instruct T cell responses against pathogens remains unclear. In this study, we investigate whether vitamin D treatment of primary human cytokine-derived DCs regulates their ability to instruct pathogen-specific T cell responses. Consistent with previous findings, vitamin D-DCs show a semi-mature phenotype characterized by intermediate expression of antigen-presenting and co-stimulatory molecules such as HLA-DR, CD1a, CD80 and CD40. However, vitamin D-DCs are slightly superior in promoting pathogen-specific T cell proliferation *in vitro*. Furthermore, we find that vitamin D treatment during DC differentiation significantly promotes secretion of pro-inflammatory cytokines, such as TNF-α, IL-6 and IL-1β in response to different pathogen-associated molecular patterns. Strikingly, the cytokines secreted by vitamin D-DCs promote the differentiation of Th22 cells, which regulate cutaneous immunity, in part by triggering expression of antimicrobial peptides in keratinocytes via IL-22 secretion. In summary, our study suggests an unexpected effect of tolerogenic vitamin D-DCs in promoting T cell-mediated host defense in the skin and may further our understanding how vitamin D modulates T cell immunity in humans.

P209

Utilization of a PMBC transfer model into immunodeficient mice to study human T cell responses against *L. major* infection

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Immunodeficient mice as recipients for human immune cells are a powerful tool to analyze human immune responses *in vivo*. The transfer of human peripheral blood mononuclear cells (PBMC) leads to reconstitution of immune-deficient mice with human CD8+ and CD4+ T cells. To analyze human T cell responses against *Leishmania major* (*L. major*) infections, we applied this model by transfer of PBMC together with injection of live parasites. As a proof of principle, to show that transferred T cells are able to mount antigen-specific responses *in vivo*, we injected 20×10^6 PBMC i.p. together with syngeneic human DCs into NOD-SCID γc−/− mice and 3 weeks later restimulated spleen cells of these mice with human syngeneic DC pulsed with OVA or infected with *L. major*. Spleen cells isolated from mice transferred with *L. major* -infected DC +PBMC released increased levels of human IFNγ when restimulated with infected DC, while cells from mice transferred with PBMC and uninfected DC combined with free parasites or i.p. infected mice +PBMC did not. To analyze the effect of human PBMC transfer over a period of 9 weeks, we assessed lesion sizes in i.d.-infected ears of NOD-SCID or NOD-SCID γc−/− mice transferred with 50×10^6 PBMC. Only NOD-SCID mice showed significantly larger lesions upon PMBC transfer compared to control mice with the tendency to develop larger lesions when a higher level of humanization was achieved (<compared to ≥10% human CD45+ cells in spleens, week 9 p.i.). Lesional parasite loads were generally high in immunodeficient- and even increased in PBMC-transferred mice. Furthermore, PBMC transfer favored visceralization with stronger parasite dissemination into spleens, especially in those mice with higher levels of humanization. Unfortunately, PBMC transfer triggered tremendous GvHD in individual mice of both mouse strains and affected survival, especially in NOD-SCID γc−/− mice. This was identified by monitoring GPT (ALT) blood serum levels in 3 week intervals and by survival; mice were sacrificed if substantial weight loss was detected. We next tested NOD-SCID γc−/− mice, known to be strongly affected by PBMC transfer if the suppression of GvHD influences lesion development. For this, we injected a Treg-activating HIV glycoprotein, gp120, together with human PBMC. We observed that gp120-administration did not affect lesion development and partially prevented GvHD in individual mice as indicated by a higher survival rate and a reduced number of mice showing strongly elevated GPT (ALT) levels. Taken together, PBMC transfer influenced lesion development, lesional parasite loads and visceralization in a disease promoting manner. The level of humanization appeared to influence the effect. Which cells and underlying mechanism lead to these effects will have to be investigated; in addition, strategies to prevent xenogeneic GvHD in this model will aid the development of a suitable model for humanized mice in cutaneous leishmaniasis and to assess therapeutic approaches.

P210

Identification of new protein targets as potential vaccine candidates against *Leishmania major* parasites

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Leishmania (L.) major parasites are responsible for the worldwide occurring disease pattern of human cutaneous leishmaniasis. It is known that both antigen-specific CD4+ and CD8+ T cells promote healing in *L. major* -infected immunocompetent hosts by releasing interferon (IFN)-γ. However, currently no vaccine against this pathogenic parasite exists. To identify antigenic proteins which could serve as vaccine candidates, we separated *L. major*-specific soluble proteins (SP) from highly immunogenic soluble Leishmania antigen (SLA, i.e. parasite lysate) by subcellular centrifugation. Next, C57BL/6 mice were immunized i.d. in one ear with different amounts of SP (ranging from 0.01 g up to 100 g) combined with CpG as adjuvant in a prime/boost/boost approach. One week afterwards, infections with live *L. major* promastigotes were initiated in the contralateral ear and lesion development was monitored weekly in three dimensions. As expected, the protective effect of SP *in vivo* was strongly dose-dependent. Immunization with 1 g SP combined with CpG induced both CD4+ and CD8+ antigen-specific T cell proliferation as determined by CFSE-labelling and flow cytometry. Thus, SP seem to contain highly potent components, which are beneficial for mediating effective immune responses against *L. major* infections. Subsequently, we aimed at identifying and characterizing single immunogenic antigens from SP. Toward this purpose, SP was fractionated by two-step anion exchange chromatography. We tested eluted protein fractions *in vitro* in a restimulation assay using draining lymph node (dLN) cells from *L. major*-infected, resistant C57BL/6 mice. dLN cells restimulated with SP showed a dominant Th1/Tc1 cytokine profile similar to that observed after incubation with total SLA. Surprisingly, only few reactive fractions from SP were found, which induced high IFN-γ levels compared to unstimulated controls. Overall, we identified 36 parasite-specific proteins in reactive fractions by label-free quantitative mass spectrometry. Next, we compared the revealed protein content with their respective reactivity profile, i.e. the fraction-specific capacity to induce high IFN-γ production, and then chose four protein candidates (protein A, C, D and E) to be subjected to further analysis. The quantitative occurrence of these proteins is mainly conforming to the dominant Th1/Tc1 cytokine profile of high reactive fractions. Currently, two out of four selected proteins which were recombinantly expressed in *E. coli* are tested in immunization studies *in vivo* for their protective capability in terms of vaccination against *L. major*. This approach revealed first promising results as C57BL/6 mice immunized with 10 g of protein C or a pool of protein A and C (10 g of each protein), respectively, showed smaller lesion sizes p.i. in contrast to mice treated with 10 g of protein A + CpG or negative control groups. The chosen antigenic vaccine candidates may serve as potential sources for protective T cell epitopes and may aid a better understanding of the underlying T cell-mediated healing processes in infected individuals or those at risk.

P211 (O26)

Intracellular TLR signalling in *L. major*-infected dendritic cells is responsible for the generation of protective immunity

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Infected, skin-derived dendritic cells (DC) are critical for the development of protective immunity against murine experimental *L. major* infection, which in turn is dependent on the efficient induction of IFN?-producing Th1/Tc1 cells. In contrast to macrophages, in which CR3-mediated phagocytosis leads to a silent invasion, parasite internalization by DC is promoted by FcγRI/III and leads to cell activation with subsequent (cross-)presentation of Leishmania-derived antigens to CD4+ and CD8+ T cells. The role of Toll-like receptors (TLR) in this process remains unclear. Prior work demonstrated that MyD88−/− mice on a genetically resistant C57BL/6 background develop progressive disease, whereas IL-1RI (and RII)−/− developed self-healing lesions similar to wt. To dissect the contribution of individual TLR to enhanced disease susceptibility, we first generated bone marrow (BM)-derived DC from various TLR ko strains and studied parasite uptake and cytokine release. Interestingly, although infection rates of all DC were similar and independent of TLR signalling, subsequent IL-12p40 release was significantly reduced by >80% in MyD88−/−, TRIF−/− and TLR9−/− DC, while other cytokines were not affected. In contrast, no effect on IL-12p40 production was seen in TLR2, TLR4 and TLR7 knockout BMDC. Next, MyD88-, TLR2-, TLR4- and TLR9-deficient C57BL/6 mice were infected with physiological low dose inocula of *L. major* mimicking natural parasite transmission. Lesion sizes and parasite loads assessed over several weeks revealed that both MyD88−/− and TLR9−/− exhibit progressive disease and succumb to infection around week 9, whereas all other strains tested healed within 12 weeks similar to wt. Finally, we hypothesized that TLR/MyD88 signalling in DC V but not other cells V is responsible for efficient protection against *L. major* infection. To this aim, we generated transgenic mice that express MyD88 exclusively in CD11c+ or in Langerin+ DC. Intriguingly, only those mice that harboured CD11c+ DC competent in MyD88-mediated TLR signalling were able to contain the infection, whereas mice with MyD88 signalling limited to Langerin+ DC developed progressive disease comparable to MyD88−/− and TLR9−/−. In summary, we establish that TLR9 signalling in *L. major* -infected DC via MyD88 and TRIF is critical for the generation of protective immunity against this important human pathogen. Thus, TLR9 on DC may be an attractive target for therapeutic strategies in vaccination trials against *Leishmania* spp.

P212

Differential contribution of Ly6-C and Ly6-G+ cells to disease outcome in experimental *L. major* infections

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Skin macrophages (MΦ) play an important role for the host defence against physiologically relevant low dose *L. major* infections. First, in the early phase, CR3-mediated phagocytosis leads to a 'silent invasion' of the MΦ, resulting in parasite transformation and replication with no apparent inflammation. Next, after 3–4 weeks, an inflammatory response is initiated in which MΦ-derived IL-6 may play a role. Other phagocytes, such as neutrophils, dendritic cells (DC), B cells, followed by T cells are recruited. In this phase, antigen presentation of infected DC to T cells induces an efficient protective response in resistant mice mediated by IFNγ release from Th1/Tc1 cells. IFNγ in turn activates infected MΦ to produce NO and to eliminate the parasite. In susceptible BALB/c mice, aberrant development of Th2/Th17/Treg development is associated with an inability to kill the parasite. To date, the precise contribution of MΦ to this process and the strain dichotomy is not fully understood. First, mice were infected with 10E3 infectious stage *L. major* and immigration of Ly6-C/-G-expressing cells was monitored over time in resistant C57BL/6 compared to BALB/c mice. Interestingly, starting in wk6, the frequency of CD11b+ myeloid cells was much higher in BALB/c lesions compared to C57BL/6. Among these, a striking strain difference was observed: BALB/c lesions contained significantly more Ly6-Cint/Ly6-Ghigh cells (~5-fold), confirming prior data, but also 2,5-fold more Ly6-Cneg/Ly6-Gneg cells. Interestingly, 6-colour flow cytometry revealed that these cells were also MHC II+ (~60%), F4/80+ (25%), CD11c+ (20%) and Gr-1+ (20%). In contrast, C57BL/6 lesions harboured many more Ly6-Chigh/Ly6-Gneg cells starting in wk6 post infection. Expression of F4/80 in this subset was found on ~20% of cells, whereas MHC II was found on 40-60%, CD11c on <5% and Gr-1 on 30–50%. Finally, Ly6-Cint/Ly6-Ghigh neutrophils exhibited in ~15% expression of F4/80, MHC II or CD11c, whereas Gr-1 was found on almost all cells. We thus classified Ly6-Cint/Ly6-Ghigh cells as neutrophils, Ly6-Cneg/Ly6-Gneg cells as DC/MΦ, both of which were more abundant in BALB/c lesions, whereas lesions of C57BL/6 mice comprised more Ly6-Chigh/Ly6-Gneg cells characterized as inflammatory monocytes (Mo)/myeloid-derived suppressor cells (MDSC). Next, to assess the exact contribution of F4/80 to disease development, we infected C57BL/6 F4/80-deficient mice with physiologically relevant low dose inocula. Lesion sizes were measured for several weeks. Interestingly, mice deficient in F4/80+ ΦM exhibited worsened disease outcome with larger lesions, but, unexpectedly, healing was nevertheless observed after several weeks. A detailed analysis of the MΦ compartment in F4/80+ mice is currently underway as is the resulting immune response. In summary, our results indicate that CD11b+ myeloid cells contribute significantly to disease outcome in cutaneous leishmaniasis. A clear cut understanding of the contribution of each subset found in skin lesions may aid development of improved therapeutic strategies.

Pharmacology

P213

Topical application of endocannabinoid modulators leads to reduction of epidermal proliferation and induction of differentiation in mouse skin

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Endocannabinoids are derivatives of arachidonic acid, part of the nerve system and exhibit neuromodulatory effects. In addition, they are important regulators of cell signalling in the body. Recently, it has been shown that cannabinoid receptors are involved in keratinocyte proliferation and differentiation and that the elevation of the endogenous cannabinoid tone leads to strong anti-inflammatory effects (Karsak et al, Science 2007; Roelandt et al, Exp. Dermatol, 2012; Lotts et al, Exp Dermatol 2012; Vasas et al, J Invest Dermatol 2013). Here we explored the influence of two endocannabinoid modulators, a selective fatty acid amide hydrolase (FAAH) inhibitor WO20100083-440 (WO440) and WOL067-531 (WO531), a selective inhibitor of endocannabinoid reuptake, compared to hydrocortisone(17-)butyrate (HC-B), on skin permeability barrier repair, epidermal proliferation, differentiation and antimicrobial protein expression in hairless mice. Barrier disruption was induced by tape-stripping. Immediately after barrier disruption 30 microliter of a solution of WO440, WO531 (0.5% and 0.05%), HC-B (0.1%, which equals the concentration of the commercial cream preparation) or the vehicle (isopropanol/ propylene glycol, 3:7 v/v) were applied topically. Barrier repair was monitored by measurements of the transepidermal water loss (TEWL) for 24 h. At 24 h skin biopsies were obtained and histology and immuno-histology with antibodies for Ki-67, involucrin, loricrin, filaggrin, mouse beta-defensin (mBD) 1, 3 and 14 and CRAMP were performed. WO531 (0.5%) and HC-B led to a significant delay of the permeability barrier repair at 1.5, 3, 5, and 7 h. At 24 h no significant differences were found any more. Application of WO440 (0.5%) resulted in a delay of the barrier repair but without statistical significance. The 0.05% concentrations of WO440 or WO531 did not influence barrier repair significantly. Histology and immuno-histology showed that barrier disruption by tape-stripping led to an increase in epidermal proliferation and epidermal thickness (acanthosis), changes in epidermal differentiation and an increase in the expression of antimicrobial proteins. The increase in epidermal proliferation and epidermal thickness was reduced by topical applications of WO440, WO531 and HC-B. Also, the changes in the expression of the differentiation marker loricrin after barrier disruption were reduced by WO531. In parallel with the induction of differentiation the increased expression of mouse beta-defensins mBD-3 and mBD-14 after barrier disruption was reduced by WO440, WO531 (0.5%) and HC-B. Involucrin and filaggrin as well as mBD-1 and LL37 expression were not significantly influenced by barrier disruption or by application of the compounds. In summary, we showed that topical application of WO531 and HC-B after experimental skin barrier disruption resulted in a transient delay in skin barrier repair, reduction of epidermal hyperproliferation, reduction of an increase in epidermal skin thickness, induction of differentiation and normalization of defensin expression. This may be related to the known anti-inflammatory effects of endocannabinoids and corticosteroids. Endocannabinoid modulators show similar acute effects as a corticosteroid and might be useful drugs to control inflammation, epidermal hyperproliferation, acanthosis and differentiation in diseases like contact dermatitis or atopic dermatitis.

P214

Antimicrobial properties of sap obtained from fresh leaves of *Isatis tinctoria* determined by microplate laser nephelometry

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Objective: Woad, *Isatis tinctoria* L. (Brassicaceae) is an ancient plant that was used to produce the blue dye indigo but also as a medicinal plant for centuries. Because of its antiinflammatory, antiviral and possible anticarcinogenic properties, woad is still applied in folk medicine. Moreover a strong preservative effect against fungal decay has been observed. Active compounds such as tryptanthrin, indole-3-acetonitrile and p-coumaric acid methylester have been shown to exhibit antimicrobial activity against bacteria, yeast and dermatophytes. Hence, it is of great interest to investigate the antimicrobial effect of woad especially for its application in formulations for adjuvant treatment of wounds and skin diseases. In this present study we determined the antibacterial and antifungal activity of sap obtained from fresh woad leaves against bacteria like *Staphylococcus aureus* and *Klebsiella pneumoniae* and yeast such as *Candida albicans* and *Malassezia pachydermatis*. Due to the complexation capacities of cyclodextrins (CDs) for diverse molecules, they are able to protect their 'guests' from degradation but might also influence their physiological distribution. Thus, we further tested various CDs-complexes of woad sap for antifungal activity.

Method: The antimicrobial activity of sap obtained from fresh woad leaves as well as alpha-, beta and gamma-CD complexes of woad sap against *S. aureus* ATCC 6538; *K. pneumoniae* ATCC 4352; *C. albicans* DSM 1386 and *M. pachydermatis* DSM 6172 was analysed *in vitro* by microplate laser nephelometry (MLN) using the NEPHELOstar Galaxy (BMG LABTECH, Ortenberg, Germany).

Results: It could be demonstrated that sap of fresh woad leaves alone showed a significantly higher antibacterial capacity against the gram-negative bacteria *K. pneumoniae* than against the gram-positive bacteria *S. aureus*. The complexation of woad sap with alpha-CD significantly increased the antibacterial effect against both bacteria species (IC50 = 10.6% vs. 6.49%; 18.1% vs. 15.08%), while beta- and gamma-CD only lead to a small increase of the efficiency. Moreover, woad sap alone exhibited a strong fungicidal activity against *C. albicans* (IC50 = 1.92%) and *M. pachydermatis* (IC50 = 1.32%) at low concentrations. Likewise, the complexation with alpha- and beta-CD significantly increased the fungicidal properties especially against *C. albicans* (IC50 (alpha) = 0.33%, IC50 (beta) = 1.11%) but also for *M. pachydermatis* (IC50 (alpha) = 1.31%, IC50 (beta) = 1.18%). In contrast, complexation with gamma-CD did not affect the fungicidal activity of sap from fresh woad leaves.

Conclusions: These *in vitro* experiments demonstrate a strong antimicrobial capacity of sap from fresh woad leaves against tested bacteria and yeast. In addition, complexation with CDs of smaller molecule size resulted in an increase of the antimicrobial activity of woad sap. These results are crucial evidence that woad could be a natural source of antibacterial and antifungal agents.

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Dimethylfumarate and monomethylfumarate regulate indolamin 2,3-dioxygenase activity and expression in peripheral blood mononuclear cells

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Indolamine 2,3-dioxygenase (IDO) is a heme-containing, rate limiting enzyme, which catalyze the conversion of tryptophan to kynurenine, the main tryptophan metabolite. The expression of IDO can be induced by the pro-inflammatory cytokines mainly interferon-gamma (IFNγ), interleukin-1 (IL-1) and tumour necrosis factor alpha (TNFα). IDO has a highly complex role in immunoregulation, infection, autoimmunity, transplantation and cancer. It has been shown that IDO is activated in a number of patients with psoriasis and that IDO activation correlated with more extensive disease.

Fumaric acid esters (FAE) have been used for the systemic treatment of psoriasis since 1959. Moreover, clinical interest in FAE is not limited to psoriasis but was extended to other immune-mediated inflammatory diseases such as multiple sclerosis. Interestingly, orally administered dimethylfumarate (DMF) reduced melanoma growth and metastasis in mouse models. We have previously shown that, treatment of melanoma cells with DMF reduced IDO-activity and expression. However, the relevance of IDO and the effects of FAE on IDO activity in psoriasis have not been investigated. Therefore, we have studied the effect of DMF and its metabolite monomethylfumarate (MMF) on the expression and activity of IDO in peripheral mononuclear blood cells (PBMC). PBMC were treated with different concentrations of DMF or MMF for 30 min and thereafter stimulated with 100 ng/ml IFNγ for 24 h. IDO activity was determined by measuring the concentration of kynurenine in the culture medium employing a HPLC technique. IDO-protein expression was determined by Western blot. The results of the study show that, DMF and MMF inhibit IDO activity and expression in PBMC. In addition, we have shown for the first time that, MMF has an inhibitory effect on IDO-activity *in vitro* in therapeutic concentrations.

With this study we want to contribute to the understanding of the mode of action of DMF and its metabolite MMF. The results of this study imply that DMF and MMF regulate IDO activity in a dose dependent manner in immune cells and may partly explain the therapeutic activity in psoriasis and other fumarate-sensitive disorders.

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Wound healing studies with Traditional Chinese Herb 22 in an *ex vivo* porcine skin model

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Chronic wounds generate severe health care problems worldwide with a high burden for the patients and high costs for the health care system. They are the result of dysregulated wound regeneration due to various causes, including vascular and metabolic diseases or infection. Often, no satisfactory treatment is available. In the course of developing new therapy strategies to heal chronic wounds and to improve wound healing in general, a reinvestigation of long used therapies, including Traditional Chinese Medicine, is a promising strategy. This investigation leads to better understanding of the mechanisms of these therapies and an optimization of their application.

The Chinese Herb analyzed in this project (Chinese Herb 22 (CH22)) is traditionally used for the treatment of eczema, ulcer and burn wounds. It has been shown that extracts of CH22 have antimicrobial and antioxidative effects as well as a positive impact on healing of burn wounds and fibroblast viability in rats. However, often the extracts used are not well defined and no reports are available that analyze the impact of CH22 on human cells. For a better understanding and further treatment optimization we investigated the effect of a water and an ethanol extract of CH22 on cultured keratinocytes and an *ex vivo* porcine wound model with defined mechanical wounds. The water extract of CH22 shows cytotoxicity at higher concentrations in MTT assay. At lower concentrations, there was no impact on cell viability with both extracts. In the *ex vivo* model we saw a significant positive wound regenerating effect of the water extract of CH22 in comparison to the placebo control. We further stratified the results into two groups concerning their donor intrinsic healing capacity (good and bad healing wounds), where we observed a general positive effect for both groups but especially a significant wound healing impact on skin with impaired wound healing.

These data indicate a positive wound healing effect of CH22 in general as well as a significant influence on impaired healing skin, with a promising outlook for the treatment of chronic wounds.

P217

2-Methoxyestradiol impairs lymphangiogenesis through G2/M cell cycle arrest and apoptosis

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Lymphangiogenesis is a crucial step in the progression of cancer. Formation of new lymphatic vessels provides an additional route for tumor cells to metastasize. Therefore, inhibiting lymphangiogenesis represents an interesting target in cancer therapy. 2-Methoxyestradiol (2-ME) is a physiological metabolite of 17β-estradiol that appears to have potent anti-proliferative effects on various tumor cells. 2-ME is characterized by low cytotoxicity and could be a valuable anti-tumorigenic molecule for treating cancer. As 2-ME promotes anti-angiogenic effects on endothelial cells, we hypothesized that 2-ME may have impact on lymphangiogenesis. To prove this assumption, we first performed proliferation assays with primary human lymphatic endothelial cells (LEC). 2-ME inhibited cell proliferation in a concentration-dependent manner. To further elucidate the underlying anti-proliferative mechanisms of 2-ME, we performed cell cycle FACS and apoptosis analysis. We found that 2-ME induced both G2/M arrest and apoptosis in LEC. Cell cycle arrest was accompanied by up-regulation of p53 and p21, as well as down-regulation of Cyclin B1, Cdc25c and cdc2. In addition, 2-ME induced apoptosis by cytochrome c release, activating Caspase-9, -7 and -3 and cleavage of poly ADP-ribose polymerase (PARP), whereas cleavage of Caspase 8 was unaffected by higher concentrations of 2-ME. Furthermore, the pro-apoptotic Protein Bim was up-regulated after 2-ME treatment. Kinetic studies revealed that 2-ME induced in a time-dependent manner an activation of extracellular signal-regulated protein kinase 1/2 (ERK1/2) and c-jun N-terminal kinases (JNK) in LEC. Inhibition of JNK and ERK1/2 by SP600125 and PD98059 reduced 2-ME-induced apoptosis of LEC. In further analysis, we could demonstrate an inhibition of the formation of capillary like structures by 2-ME treatment. In conclusion, we demonstrate that 2-ME has distinct anti-lymphangiogenic effects. This action seems to be mediated by cell cycle arrest and activating the intrinsic apoptotic pathway.

P218

Anti-inflammatory activity of clotrimazole on TPA- and oxazolone-induced ear swelling in mice

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Introduction: Fungal infections are frequently accompanied by inflammation of the affected regions. Imidazole drugs are not only effective in eliminating fungal infections, but also in rapidly reducing the concomitant inflammation, presumably by down-modulating the expression of proinflammatory cytokines.

The imidazole derivate clotrimazole (CLT) has been in clinical use for more than 25 years and has been demonstrated to inhibit trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats and tumor necrosis factor (TNF)-alpha-induced adhesion molecule expression *in vitro*.

Methods: We investigated the anti-inflammatory potency of CLT in two acute inflammation models. Therefore, skin inflammation was induced in female BALB/c mice by topically application of 12-O-tetradecanoylphorbol acetate (TPA) and oxazolone (OXA) on one ear, respectively. 15 min. thereafter, CLT in various concentrations was applied and the resulting ear edema was quantified by measuring the increase of ear thickness after 24 h.

Results: While CLT had no significant impact on OXA-induced ear inflammation, it reduced the TPA-induced ear swelling dose-dependently and significantly up to 43.6% (CLT 2%). Moreover, even at concentrations below 0.1% a significant reduction in ear edema could be observed.

Conclusion: Our observations suggest that CLT possesses pronounced anti-inflammatory activities and selectively suppresses inflammatory pathways that may also be relevant for the concomitant inflammation commonly seen in fungal skin infections.

P219

NRF2-activation in DC protects from Th1/Th17-mediated autoimmune disease

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A number of novel oral compounds have been established for the treatment of multiple sclerosis (MS) and psoriasis. Psoriasis, MS and associated murine models of autoimmune diseases like experimental autoimmune encephalomyelitis (EAE) are characterized by the activation of autoreactive CD4-positive T helper (Th) cells. Such autoreactive Th cells typically demonstrate an interleukin (IL-)17 (Th17) and interferon (IFN-)γ (Th1) dominating phenotype. We have shown previously that the small molecule dimethylfumarate (DMF) can protect from psoriasis and EAE by suppressing IL-23 and IL-12 expression in dendritic cells (DC). By regulating the cytokine expression of DC DMF inhibits the development of autoreactive Th17 and Th1 cells. The control of cytokine expression in DC by DMF could be linked to the transcription factor NRF2. To show that NRF2 activation in DC is a feasible mechanism for suppressing autoreactive Th17 and Th1 responses we now studied the effects of a natural NRF2-activating compound on the immune system. Mice treated with this natural compound were protected from severe EAE when immunized with myelin peptide in CFA. Disease amelioration was accompanied by a reduction of IL-17 and IFN-γ expression in peripheral CD4 T cells and in CNS-infiltrating T cells. At early time points after immunization we found a suppression of IL-23 and IL-12 *in vivo* only in mice treated with the NRF2-activating natural compound. Extensive *in vitro* studies confirmed the activation of NRF2-related genes in DC and the timely regulation of certain inflammatory genes. Importantly, when DC were treated with the natural compound and activated with LPS, we found a suppression of IL-12 and IL-23 production, while no changes were found in IL-10 or IL-6 production. In contrast, the compound had no direct effects on cytokine expression by T cells in the absence of DC. By gene expression studies, reporter assays and chromatin immunoprecipitation we could further elucidate the molecular events altering the transcriptional regulation of IL-23 and IL-12 in DC. Thus, NRF2 activation by a natural compound is an elegant approach to inhibit inflammatory Th17 and Th1 responses and autoimmune pathology.

Photobiology

P220

Singlet oxygen luminescence in a polyurethane matrix and its phototoxic efficacy on *S. aureus*

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Rising resistance of bacteria against antibiotics requires new strategies for disinfection. In order to avoid spread of bacteria via contaminated surfaces, it should be a promising procedure to create self-disinfecting coating of such surfaces that are able to generate reactive oxygen species (ROS) such as singlet oxygen. This goal can be approached by attaching photosensitizers to surfaces via coating or by adding them to the bulk material. The photodynamic procedure may start whenever ambient or artificial light is shining on such primed surface. As a result, singlet oxygen is generated via type-II-mechanism close to the surface that may escape from surface by diffusion, may reach the attached bacteria and may induce non-specific oxidative damage of bacteria.

In this work a plate of poly-methyl-methacrylate (PMMA) was coated with a thin layer of polyurethane (PU) with a thickness $\approx$ 30 μm that contained the porphyrin meso-Tetraphenylporphyrin (TPP). We investigated the photophysical and the photodynamical properties of that surface. The photostability of the photosensitizer and its potential leakage from PU was analysed with absorption spectroscopy. Singlet oxygen was generated by irradiating the probes with an OPO tuneable laser at λ = 420 nm in the Soret band of the photosensitizer. Singlet oxygen was detected directly by spectral and time resolved measurements at 1270 nm in near-backward direction with respect to the exciting beam using an infrared-sensitive photomultiplier. In order to investigate the singlet oxygen escape from the PU surface, the technique of the generation of triiodide by the reaction of potassium iodide with singlet oxygen was applied. The photodynamic inactivation of bacteria on the surface was investigated with the gram-positive *Staphylococcus aureus*. Thus, after drying a drop of a bacteria suspension on the surface, the plate was irradiated with an appropriate incoherent light source for 10 and 30 min with 50 mW cm(-2) (wavelength range 400–800 nm). The emission spectrum of the used incoherent light source closely matched the absorption peaks of TPP.

No leakage of the photosensitizer into water surrounding was detected. PU is gas permeable and a sufficient amount of oxygen reaches the photosensitizer. This enabled singlet oxygen generation that was directly proven by the detection of its luminescence. The rising and decaying part of the luminescence signal were evaluated. The obviously low quenching rate constants of PU matrix yielded a lifetime of singlet oxygen that was longer as compared to pure water. In order to clarify which part of the luminescence signal referred to singlet oxygen decay, experiments were carried out at different oxygen concentrations. Furthermore we detected singlet oxygen diffusing from the surface into the surrounding by the formation of triiodide in aqueous suspension.

To prove photodynamic inactivation of bacteria on the PU surface, we investigated two different concentrations of the photosensitizer and two different applied light doses. A photodynamic killing of ≥99% (2 log10 steps) was achieved by an applied light dose of 90 J cm(-2) with a photosensitizer concentration of 2o10(-4) M.

We showed that singlet oxygen is generated in such PU surfaces and that a killing of *S. aureus* with an efficiency of 99% was possible with the given properties. Further investigations will clarify if the efficacy can be enhanced using other photosensitizers and irradiation procedures.

P221

Singlet oxygen generated by skin care products and their ingredients via UVB excitation

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Cellular damage through UV radiation has been assigned either to direct DNA damage caused by UVB (280–320 nm) radiation or to UVA (320–400 nm) damage via endogenous photosensitizers that generate singlet oxygen. Recently, it was shown that also UVB radiation is able to generate singlet oxygen via endogenous photosensitizers. In addition, some endogenous molecules like α-tocopherol (vitamin E) are often used as ingredients in skin care products. Skin care products are applied topically and can penetrate the skin to a certain extent. If a skin care product generates singlet-oxygen via UV excitation, singlet oxygen might damage skin cells upon UV-exposure.

In the present study we aimed to investigate whether different skin care products are able to generate singlet-oxygen by UVB or UVA excitation. Singlet oxygen is directly detected and quantified by its luminescence in the near infrared spectrum at 1270 nm.

First of all, the singlet oxygen generation of α-tocopherol was investigated for excitation in the UVB range. A clear singlet-oxygen signal could be detected with a quantum yield of 15 2%. That is, about 15% of absorbed UVB radiation is converted to singlet oxygen. Subsequently thirteen different skin care products and four sun screens were investigated. For all investigated sun screens no singlet oxygen luminescence signal could be detected. But for eight of the investigated skin care products a clear time and spectral resolved singlet oxygen luminescence signal could be obtained via UVB or UVA excitation.

In conclusion, some skin care products may contain substances, which are photosensitizers yielding hazard singlet oxygen upon UV excitation. In the near future, singlet oxygen generation of these skin care products will be investigated while applied on skin and exposed to UV radiation, in particular using solar radiation.

P222 (O11)

Downregulation of miR-15b is associated with increased sirtuin 4 (SIRT4) expression in premature fibroblast senescence in-vitro and photoaging of human skin *in-vivo*

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Extrinsic aging is associated with DNA damage and mitochondrial dysfunction. Sirtuins are deacetylases or ADP-ribosyltransferases which are implicated in metabolism and life-span regulation. All seven sirtuins are expressed in human keratinocytes, fibroblasts *in vitro*, and in skin *in vivo*. Previous studies employing various premature senescence models using MCF7 cells gene-array based expression screens which included all seven sirtuins indicated a prominent role of SIRT4 in cellular senescence, because this was the only consistently and significantly induced sirtuin. Since (i) SIRT4 is known to negatively impact on mitochondrial oxidative capacity and (ii) disturbed mitochondrial function of dermal fibroblasts is thought to be of major pathogenic relevance for photoaging of human skin, we hypothesized that dysregulation of SIRT4 expression may be linked to photoaging of human skin. We found that SIRT4 mRNA levels were significantly increased *in vitro* in human dermal fibroblasts (i) as a function of increasing passage number, i.e. replicative senescence, or (ii) after repetitive UVB exposure or treatment with ionizing radiation, i.e. stress-induced senescence. The *in vivo* relevance was assessed by comparing skin taken from young (18–25 years) and older adults (60–66 years) both from sun-exposed neck and from sun-protected buttock. Accordingly, *in vivo*, SIRT4 mRNA levels were significantly up-regulated in chronically sun exposed skin from the neck versus intrinsically aged skin from the buttock of volunteers with older age ($n = 16$). Analysis of enzymatically separated skin samples indicated that increased SIRT4 expression was mainly confined to the dermis. In corresponding skin samples from younger adults ($n = 15$) no such differences were observed.

As microRNAs (miRNAs), i.e. small genome-encoded, about 22 nucleotide-long RNAs that silence gene expression post-transcriptionally by binding to 3′-untranslated regions of messenger RNAs, have been implicated in various senescence and aging models, we next performed a global microarray based comparison of corresponding miRNA- and mRNA expression profiles in MCF7 cells. By validation in various *in vitro* senescence models including human fibroblasts treated with ionizing radiation we consistently observed that a significant up-regulation of SIRT4, a bona fide target of miR-15b, was associated with significantly decreased levels of miR-15b. Also, in human skin, highest miR-15b copy numbers were detected in the epidermis, and epidermal expression was significantly reduced in photo-aged skin versus intrinsically aged skin. Reduced miR-15b expression is most likely causally linked to increased SIRT4 expression because we could show that (i) miR-15b targets a conserved binding site within the 3′-untranslated region of the SIRT4 gene as demonstrated by luciferase reporter assays and (ii) transfection of oligonucleotides mimicking miR-15b function was sufficient to prevent SIRT4 up-regulation in senescent cells. Thus, miR-15b may act as a negative regulator of SIRT4 expression to antagonize mitochondrial dysfunction and hence cellular senescence and tissue aging, particularly photo-aging of the skin.

P223

Sensing of UVB-induced DNA damage in the skin by the innate immune system involves the TLR4- and MyD88-dependent signaling pathway

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Background: Excessive exposure of the skin to UVB radiation causes DNA damage in epidermal keratinocytes and initiates a reactive neutrophil-rich inflammatory response. Little is known how the innate immune system senses UVB-induced DNA damage in the skin. We hypothesized that TLR-dependent signalling pathways participate in this process.

Methods: We investigated how genetic or pharmacologic blockade of TLR signalling impacts upon skin inflammatory responses induced by two consecutive sunburning doses of 4.5 kJ/m^2 UVB on the back skin.

Results: Histopathological analyses revealed reduced numbers of infiltrating immune cells and diminished reactive hyperproliferation of epidermal keratinocytes in UV-irradiated skin of Tlr4−/− and MyD88−/− mice, but not of Tlr3−/− and Trif−/− mice, when compared to wild-type controls. Flow cytometric and gene expression analyses confirmed that the systemic activation and local recruitment of neutrophils was largely absent in Tlr4−/− and MyD88−/− mice but not in Tlr3−/− and Trif−/− mice. The reactive skin inflammatory response following UV irradiation was also largely abrogated by the TLR4 inhibitor CLI-095 or by antibody-mediated depletion of neutrophils.

Conclusions: Our results demonstrate that the TLR4- and MyD88-driven innate immune signalling pathway critically contributes to the reactive neutrophil-rich inflammatory response following UVB-induced DNA damage in the skin.

P224

Analysis of the interplay between the innate immune system and environmentally induced aging

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The skin is the largest barrier organ and protects from exogenous insults such as pathogens, but also from environmental insults or UV irradiation. Chronic exposure to UVB-irradiation leads to immunosuppression and premature skin aging. We aim to analyse whether activation of the innate immune system contributes to premature skin aging.

Toll-like receptors (TLR) recognize pathogens and sterile danger signals. The central adaptor protein for signal transduction of most TLR and the IL-1 receptor family cytokines is MyD88. Premature skin aging was induced by a six week chronic UVB irradiation model *in vivo* in MyD88-deficient and control mice. Chronic UVB irradiation leads to epidermal hyperplasia and skin tanning. In addition chronic UVB irradiation reducedabsolute numbers of Langerhans cells in the epidermis, whereas dermal mast cell numbers were significantly enhanced. Also cell numbers of skin draining lymph nodes were enlarged, but the frequencies of the different cell types like dendritic cells, B cells and T cells remained unchanged. MyD88-deficient mice showed reduced epidermal thickening and reduced dermal mast cell numbers compared to wildtype mice. In addition the enlargement of the skin draining lymph nodes after UVB irradiation as well as augmented storage of melanin in the epidermis is dependent on MyD88 signaling. These data clearly indicate that MyD88 contributes to UVB-induced skin aging *in vivo*.

Furthermore we used conditional mouse strains to investigate the cell type-specific role of MyD88-signaling during extrinsic skin aging. Therefore we generated mice which express MyD88 selectively in cells of the skin which contribute to premature aging such as keratinocytes, or in innate effector cells like macrophages and neutrophils. First results show that the expression of MyD88 selectively in macrophages and neutrophils does not contribute to epidermal thickening, but partly restores the recruitment of the mast cells into the dermis. Interestingly, expression of MyD88 in either macrophages and neutrophils or keratinocytes contributes to elevated cell numbers in skin draining lymph nodes after chronic UVB irradiation *in vivo*.

Following this approach, we aim to provide insights into the regulation of premature skin aging through signaling pathways of the innate immune system.

P225 (O07)

Bad bugs – no drugs? Vitamins kill multiresistant bacteria within seconds by light activation

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The increasing emergence of multiresistant bacteria like Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the most important clinical challenges. The pace of antibiotic development to struggle against these bacteria has dramatically dropped down and this is a worldwide worrying evolution. Besides the development of novel antibiotics, other methods for effective killing of pathogenic bacteria have been considered. One of these methods is the photodynamic inactivation of bacteria (PIB).

The mechanism of action of PIB is based on a non-toxic dye, termed photosensitizer (PS), molecular oxygen and visible light (400–700 nm). After PS excitation by light, reactive oxygen species (ROS) like superoxide anions or singlet oxygen are formed, which leads to irreversible oxidative damage of bacterial cell wall structures, proteins and DNA. Natural substances such as vitamin B convert up to 50% of absorbed light energy into highly reactive singlet oxygen. We added positive charges to vitamin B structure that the molecules can eagerly attach to the negatively charged surface of the bacteria.

For our study we selected two different derivative classes of Flavin molecules. Flavin 1 was prepared by the classical Kuhn synthesis protocol, Flavin 2 was generated by directly modification of the alcohol groups of the ribose chain with lysine by Steglich esterification. Both newly synthesized photosensitizers generated singlet oxygen with a quantum yield of 0.75 0.05 (Flavin 1) and 0.78 0.05 (Flavin 2), respectively.

MRSA was incubated with different concentrations of Flavin 1 or Flavin 2 for 10 s and was subsequently irradiated with 50 mW/cm. A Flavin 1 concentration of 10 M and an applied radiant exposure of 1.5 J/cm resulted in a bacterial killing of 5 log10 orders (=99.999%). Using 50 M of Flavin 2 and an applied radiant exposure of 1 J/cm, the number of viable MRSA substantially decreased up to 6 log10 steps (≥99.9999%), equivalent to high level disinfection. Similar results were achieved when enterohemorrhagic *Escherichia coli* (EHEC) HUSECO41 (O104:H4), multiresistant *Pseudomonas aeruginosa* and multiresistant Acinetobacter baumannii were irradiated with our new Flavin molecules. The effect was independent of the type of bacteria and its antibiotic resistance pattern due to the oxidative effect of singlet oxygen.

Additionally the cell toxicity of Flavin 1 and Flavin 2 was tested against normal human epidermal keratinocytes (NHEK). NHEK cells were incubated with Flavin 1 or Flavin 2 with concentrations up to 100 M and irradiated with the same light parameters as used for PIB. The results of the MTT assay clearly showed that cell viability was not affected by both photosensitizers for radiant exposures up to 9 J/cm (Flavin 2) or 12 J/cm (Flavin 1).

In conclusion, multiresistant bacteria can be effectively killed by a clever combination of modified vitamin B molecules, visible light and oxygen. Our new photosensitizers are vitamin derivatives and the molecules as well as associated decomposition products are common compounds in nature. Therefore, these photosensitizers should be applicable for disinfection not only in medicine, but also in food industry and many environmental technologies. In addition, PIB with both Flavin derivatives can be considered safe in humans showing a great potential of bacteria killing without harming the adjacent tissue.

P226

Blue light and UVA1 irradiation lead to reduction of T cell viability in human PBMC in a dose dependent manner

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Phototherapy is an important and effective component of dermato-therapeutic concepts especially in chronic inflammatory diseases like atopic dermatitis and psoriasis. Particularly, energetic UVB irradiation is in use but research of the last decades revealed its carcinogenic side effects. Therefore, alternative irradiation concepts with fewer side effects are strongly needed. A possible approach is the use of blue light (400–-500 nm), an UV-free device that was already shown to be clinically effective in atopic dermatitis. Since the mode of action of blue light is largely unknown, we investigated the immunologic mechanisms of irradiation with blue light and compared it to low dose UVA1. A special focus was set on T cell subpopulations.

PBMC were generated from buffy coats of healthy individuals and irradiated with either blue light (emission maximum 420 nm) or UVA1 (emission maximum 370 nm). Apoptosis and viability of T cell subpopulations was assessed at different time points after irradiation via annexin V-/ propidium iodide staining and flow cytometric analysis.

Viability of lymphocytes was significantly reduced 24 h after irradiation with subtoxic doses (14.5 J/cm2) of blue light (46.2%; $P < 0.001$) or UVA1 (33.4%; $P < 0.001$) as compared to untreated controls (100%). These effects were reflected in an increase of apoptotic cells of 132.3% for UVA1 and 66.1% for blue light, respectively. While analyzing different T cell subsets, CD3+ CD4+ T effector cells were less resistant against irradiation compared to CD4+ CD25highCD127low regulatory T cells. Remarkably, the mentioned effects are dose dependent with UVA1 being about twice as effective as blue light.

Blue light as well as UVA1 irradiation reduce the viability of T cells *in vitro* through induction of apoptosis. Referring to the doses UVA1 seems to be twice as toxic as blue light. Analysis of T cell subsets reveals that CD3+ CD4+ T cells react more sensitive against irradiation compared to CD4+ CD25highCD127low T cells. A possible reason for this effect might be the higher resistance of regulatory T cells against oxidative stress. Through augmented apoptosis of effector cells the regulatory balance among T cells could be shifted and thus could explain the immunomodulatory effect of the irradiation. Further functional analysis of treated subpopulations *in vitro* but also in *in vivo*-models are necessary to underline these first data about the mode of action of blue light irradiation on the immune system.

P227

Investigation of ultra-structural changes in multiresistant bacteria upon photodynamic treatment

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Due to the threatening evolution of multi-resistant bacteria new antibacterial strategies are indispensable. The Methicillin Resistant *Staphylococcus aureus* (MRSA) is one of the most prominent species of Gram-positive multi-resistant bacteria. In 2013 the Centers of Disease Control and Prevention (CDC) published that more than 80 000 severe MRSA infections occurred in USA. These infections include skin and wound infections that can provoke sepsis and death. More than 11 000 people died in 2011 as a consequence of MRSA infections. Approximately 8% of all healthcare-associated infections reported to CDCs National Healthcare Safety Network are caused by *Pseudomonas aeruginosa* which are Gram-negative multidrug-resistant bacteria and can induce e.g. serious wound infections.

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An innovative and very promising approach to fight multi-resistant bacteria is the photodynamic inactivation of bacteria (PIB). Here, the bacteria are incubated with dyes (photosensitizers) which are able to absorb light and to transfer charges or energy to e.g. molecular oxygen that is then converted into reactive oxygen species (ROS). These ROS can subsequently damage cellular structures such as proteins and fatty acids by oxidative stress and lead to death of these bacteria.
However, the cellular processes that occur in bacteria during and upon photodynamic treatment have not yet been fully understood. Microscopy offers the possibility to observe cellular processes in real time. Owing to their small diameter (~1 m) it is difficult to observe morphological changes by light microscopy (resolution limit ~200 nm in lateral direction). Transmission electron microscopy (TEM) offers a possibility to investigate morphological changes upon photodynamic treatment due to its higher resolution of about ~0.2 nm.
This study focuses on the investigation of ultra-structural changes upon photodynamic treatment of multi-resistant species such as MRSA and *Pseudomonas aeruginosa* that can be observed using TEM. The Gram-positive and Gram-negative bacteria, which have a different subcellular composition, were photodynamically treated using a new generation of photosensitizers. The bacterial viability was reduced by 6 log10 steps using a photosensitizer concentration of 50 M and a light dose of 3 J/cm^2 (broad band light source). The TEM images of MRSA show a destruction of the outer peptidoglycan layer as well as lamellar structures that are formed out of the cell membrane. For *Pseudomonas aeruginosa* it could be demonstrated that outer membrane vesicles are formed upon photodynamic treatment. The aim is to get a better understanding of photosensitized reactions of ROS with cellular components such as bacterial membranes and nucleic acids to further improve methodologies, in particular the efficacy of PIB by studying mechanisms of bacterial inactivation using high resolution microscopic techniques.

P228

High resolution imaging of endospores upon photodynamic treatment

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At present, many conventional antimicrobial strategies fail and there exists an increasing spread of multi-resistant microorganisms that has become a major problem not only in medicine but also in food industry. In particular, endospores of bacteria can survive harsh conditions due to the production of a multilayered and protective capsule. Thereby endospores are well armed against stress and starvation for long time periods, even at physical extremes for life forms on earth. Endopores are relatively resistant to many of the disinfectants and antiseptics such as alcohols, phenols, chlorhexidine, and benzalkonium compounds, which routinely destroy vegetative bacteria. Bacillus is a group of Gram-positive endospore-forming rod-shaped aerobic bacteria which are widely distributed in soil environments. When the bacterial endospores are inhaled, ingested, or come into contact with a skin lesion on a host, they may become reactivated. Bacillus anthracis has been recognized as a likely agent for bioterrorism because the endospores are highly stable in the environment, virtually all persons are susceptible, infection can occur as a result of inhalation of endospores, and considerable morbidity and mortality result from infection. During October and November 2001, 22 people in the United States developed anthrax (11 cases of inhalation anthrax and 11 cases of cutaneous anthrax) as a result of the intentional exposure to B. anthracis via contaminated letters. Bacillus atrophaeus has been reported to be slightly less susceptible to germicides than B. anthracis, and therefore is an excellent surrogate for our study.
Photodynamic inactivation (PDI) of pathogens is a new and very promising technique, even for the inactivation of endospores. The endospores are incubated with dyes (photosensitizers, PSs). After a short time of incubation, PSs are excited by light. This leads to the production of highly reactive oxygen species (ROS) directly at the microorganism, which oxidatively damage the protective capsule of the endospores and consequently the microorganisms are killed. However, the detailed processes of photodynamic inactivation of bacterial endospores have not yet been fully understood.
In order to investigate the photodynamic inactivation of bacterial endospores, nanometer-scale characterization of the morphological changes of the endospore structure is essential. Their endospores have a diameter of approximately 1 m. In order to visualize their ultra-structural changes upon PDI treatment, ultra-structural resolution of microscopes is needed. Transmission electron microscopy (TEM) offers the possibility to investigate morphological changes upon photodynamic treatment due to its higher resolution in the lower nanometre range (~0.2 nm).
In this study, we examined the time dependent ultra-structural destruction of Bacillus atrophaeus spores by PDI using TEM. The endospores were incubated with a new generation of flavin derivatives (photosensitizer concentration was 4 mM) and irradiated with visible light (400–700 nm) and light doses between 28 and 112 J/cm^2. After 10s of irradiation, the viability of endospores decreased by more than 6 log10 steps with a light dose of 70 J/cm^2. It could be shown that already after 8 s of irradiation the outer coat was disrupted and that macromolecules diffused out of the inner core of the endospores. We describe here for the first time the morphological changes that bacterial endospores undergo when inactivated within seconds by a new generation of food safe photosensitizers.

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Singlet oxygen measurement in skin during UV irradiation – from single molecule to complex skin structure

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UV radiation plays an important role for adverse reactions in human tissue. UV radiation penetrates epidermis and dermis (UVA only) of skin being absorbed by various biomolecules, especially endogenous photosensitizers. This may generate deleterious singlet oxygen ($1O_2$) that oxidizes fatty acids in cell membranes, lipoproteins, and other lipid-containing structures such as the epidermal barrier.
It is known today that singlet oxygen cannot only be generated by UVA excitation but also by UVB excitation in a photosensitized-driven process. Therefore different single molecules present in skin, lysed skin and intact pig skin were investigated for their ability to generate singlet oxygen under different UV excitation wavelengths.
The aim of the study was to investigate the amount of generated singlet oxygen depending on the used wavelength for excitation in solutions as well as in skin.
Endogenous molecules (e.g. linoleic acid, FMN, nicotinamid) were irradiated in solution using monochromatic UV radiation in the range from 300 to 400 nm depending on their maximal absorption. Cell lysate and pig skin were irradiated at certain wavelengths in the UV range to estimate the dependency of singlet oxygen generation vs. excitation wavelength.
Using special IR photomultipliers, singlet oxygen can be directly detected in solutions and also in skin by its extremely weak luminescence at 1270 nm. For identification of singlet oxygen, spectrally resolved luminescence measurements in the range from 1150 to 1400 nm were performed and the singlet oxygen quencher sodium azide was used.
For single molecules clear singlet oxygen signals can be detected time and spectrally resolved. Also for lysed cells and intact pig skin singlet oxygen can be detected depending on the excitation wavelength.

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Investigation of new photosensitizers for the photodynamic inactivation of bacteria *in vitro*

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Antimicrobial resistance is one of the most serious health threats in 2013. Some pathogens have turned out to be resistant to almost all classes of antibiotics. Infections with these resistant bacteria, like the methicillin resistant *Staphylococcus aureus* (MRSA), are now quite common, which is why we are interested in discovering new alternatives to fight against emerging multiresistant pathogens. The photodynamic inactivation of bacteria (PIB) is a promising new technique to inactivate microorganisms.
In this process, bacterial cells are incubated with a photoactive dye (photosensitizer, PS) and subsequently irradiated with visible light. This induces reactive oxygen species to be generated. The commonly used dye TMPyP (5,10,15,20-Tetrakis(1-methyl-4-pyridinio)-porphyrin tetra(p-toluenesulfonate)) is a porphyrin with four positive charges that has demonstrated photodynamic killing efficacy against both Gram-positive and Gram-negative bacteria. Due to the overall negative charge of the outer bacterial cell wall, positively charged moieties of an appropriate photosensitizer are a must-have. In order to improve the phototoxicity of porphyrin derivatives, a new generation of porphyrin derivatives with eight positive charges has been developed and investigated (TPyP 8+A and TPyP 8+B). The objective was to explore whether additional positive charges at the PS moiety could increase the photodynamic efficacy due to a better attachment to the negative cell wall of the bacteria.
In the course of our trial, *Staphylococcus aureus* (*S. aureus*), MRSA, *Escherichia coli* (*E. coli*), and a clinical isolate of a ciprofloxacin resistant *E. coli* (CIP) were incubated using different concentrations of TPyP 8 + A, TPyP 8 + B and – as a reference PS – TMPyP for 30 s, 10 min and 60 min each and irradiated with a broad band light source (380–700 nm) in the visible region (50 mW/cm^2) for 10 s.
In order for PIB to be considered a disinfectant, a bacteria reduction of more than 5 log10 has to be achieved. After an incubation time of 30 s, the new photosensitizers 8 + A and TPyP 8 + B showed a 6 log10 reduction of approximately 10e08 bacterial cells with a concentration of 2.5 M for all tested bacteria after illumination. In comparison, a concentration of 5 M of TMPyP was required to achieve a disinfectant effect after 30 s. After an incubation time of either 10 or 60 min, the results were in the same range for both new PS and the reference PS TMPyP: 2.5 M were required for a reduction of more than 5 log10 of all tested bacteria.
Overall, both, the new PS TPyP 8 + A as well as TPyP 8 + B showed a better photodynamic effect than TMPyP within the incubation time of 30 s. In conclusion, the photodynamic process seems to be a promising tool for clinical disinfection, as it is both fast and effective in the inactivation of multiresistant bacteria.

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Expression and regulation of the scavenger receptor OLR1/LOX1 in mammalian skin

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Damage exerted by oxidant stress in the skin and other tissues accumulates over the lifespan, and is manifested in enhanced levels of oxidatively modified lipids and proteins or as advanced glycation endproducts (AGE). Lipid oxidation within the skin is correlated to ageing and age-related pathologies that develop upon solar exposure such as actinic elastosis. As damage assoicated molecular patterns (DAMPS) oxidized lipids can be taken up by (phagocyting) cells and/or induce signaling via interaction with receptors. Evidence amounts that Lectin-like oxidized LDL receptor-1 (OLR1/LOX1) is a major receptor mediating recognition, signaling and uptake of oxidized lipids/proteins, but also dead cell remnants, AGEs; and that it mediates oxidant stress induced signaling. Most of this evidence comes from the field of atherosclerosis research, and OLR1 was so far not described in the skin, where oxidized lipids and modified protein accumulate after stress and in aging. Our preliminary data indicated that OLR1 expression is induced by stress induced senescence in human dermal fibroblasts.
In this project we investigated with immunohistochemical and molecular biological methods the expression of OLR1 in the skin of mice and humans, and its regulation by oxidative stress.
Murine back skin and ears were examined with immunohistochemistry and immunofluorescence microscopy for expression of OLR1 and its distribution within the skin. OLR1 was found expressed, with decreasing intensity, in sebaceous glands, cells tenatively identified as a mast cells, keratinocytes and dermal fibroblasts. Expression of OLR1 was verified on mRNA level in cultured fibroblasts, keratinocytes and bone marrow derived mast cells. Application of a stress protocol of two consecutive exposures to UVA (20 J/cm), H2O2 (100 M) or oxidized phospholipids led to significant induction of OLR1 expression.
This is the first description of the scavenger receptor OLR1 in cells of the skin and this allows now to study the functional contribution of OLR1 to the stress responses and aging of the skin.

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Regulation of UV induced keratinocyte apoptosis by the small GTPase Rac1

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The Rho GTPase family member Rac1 has important functions in actin cytoskeletal rearrangement as well as cell adhesion and migration. Rac1 overexpression and activity has been linked to squamous cell carcinomas of the epidermis and mucosa in humans and mice. Transgenic mice with epidermis specific inhibition of Rac1 (N17Rac1 mice) have reduced UV light induced skin cancer formation. We therefore set out to investigate the relevant mechanisms.
Mice with epidermis specific deficiency of Rac1 (Rac1-EKO) as well as, transgenic mice expressing activating and inhibitory mutants of Rac1 in the basal epidermis were generated. Both N17Rac1 mice and Rac1-EKO mice show increased keratinocyte apoptosis compared to controls upon UVB irradiation *in vivo*. The extent of keratinocyte apoptosis was, however, strongly increased in Rac1-EKO mice as compared to N17Rac1 mice. *In vitro* studies revealed that keratinocytes isolated from N17Rac1 mice are more sensitive to UVB light induced apoptosis than controls.
To analyze skin compartment specific functions of Rac1 in the protection from apoptosis we reconstituted Rac1 activity in the basal epidermal layer of Rac1-EKO mice by keratin 14 promoter driven expression of an activated Rac1 mutant, L61Rac1 (Rac1-EKO/L61Rac1 mice). This reconstitution protected keratinocytes from UVB induced apoptosis *in vitro*, showing a cell autonomous function of Rac1 in the protection of keratinocytes from apoptosis. Unexpectedly, however, reconstitution of basal Rac1 activity in Rac1-EKO/L61Rac1 mice *in vivo*, did not result in such protection, suggesting additional, non cell autonomous pro apoptotic mechanisms in these and Rac1-EKO mice. We have investigated into these mechanisms and found differential regulation of the apoptosis regulating cytokine IL-24 upon UVB irradiation in the skin of Rac1-EKO mice. We thereby provide evidence for a new, non cell autonomous mechanism of keratinocyte apoptosis which could provide new insights into the pathogenesis of sunburn and skin tumour formation.

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Induction of the progeroid/cancer prone XP-like phenotype by an antimycotic drug is mediated via reversible downregulation of DNA repair

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Prophylactic protection of patients with severe immunosuppression is of vital importance to shield the patient from opportunistic fungal infections.
It has been reported, that patients treated with a broad spectrum antimycotic drug develop adverse effects such as phototoxicity followed by pigmentary changes and the development of ultraviolet radiation (UV) associated non melanoma skin tumors. Thus, patients closely resemble the phenotype of the progeroid/cancer prone disorder xeroderma pigmentosum (XP), known to be caused by a defect in the DNA repair mechanism nucleotide excision repair (NER). So far the underlying molecular mechanisms by which this drug leads to the XP-like clinical phenotype have not been clarified. Therefore, we investigated if the antimycotic drug leads to a reduction of DNA repair and increased DNA damage. We found that long term treatment lead to suppression of unscheduled DNA synthesis as well as increased comet formation while double strand breaks, measured with neutral comet assay, were not induced. Importantly repair suppressive effects were transient since removal lead to normalization of all repair associated parameters. Furthermore, compound treatment did not cause significant transcriptional regulation of mRNA levels of NER proteins such as XPA - G, ERCC1, XAB and DNA damage signaling proteins such as Ataxia telangiectasia mutated protein (ATM). When exposed to the compound cells also did not show cell cycle arrest even in the presence of DNA damage but proliferated similar to untreated controls. Initial in silico molecular alignment indicates possible interference with sterical binding of the p53/MDM2 signaling cascade.
Taken together these results indicate that the broad spectrum antimycotic could suppress NER, increase DNA damage and thus, within months lead to photosensitivity, pigmentary changes and ultimately non melanoma skin tumors.

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Epidermal IGF signalling regulates UV-induced inflammatory responses and regeneration

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The skin is a multilayered epithelium that protects organisms from external challenges such as UV radiation. UV-induced persistent DNA damage is a primary cause for carcinogenic mutations and tumour permissive systemic immune suppression. We have recently shown that persistent transcription blocking lesions lead to attenuation of IGF-1R signalling and increased cellular resistance to oxidative stress. Moreover, epidermal loss of IGF-1R signalling reduced proliferative potential and antagonized hyperplasia induced by a carcinogenic stimulus. Here we asked how epidermal IGF-1R signalling regulates acute and long term UVB response using mice with an epidermal specific deletion of the IGF-1R (IGF-1Repi−/−). Although these mice have a thinner epidermis, UVB irradiation did not result in an increased amount of DNA damage. However, IGF-1Repi−/− mice were more susceptible to UVB-induced apoptosis. Surprisingly, despite this initial increase in cell death, UVB radiation induced an enhanced and sustained hyperproliferative response in IGF-1Repi−/− mice compared to control, as judged by interfollicular epidermal thickness, BrdU positive cells and keratin 6 staining. This is in contrast to the requirement of IGF-1R during epidermal development when loss of IGF-1R counteracts growth and proliferative potential. Further investigation showed an increased and prolonged inflammatory response as judged by increased numbers of macrophages and neutrophils in the dermis. Since newborn IGF-1Repi−/− mice showed increased total P53 protein levels and P53 is known to regulate inflammation in other systems, we next asked whether adult IGF-1Repi−/− also had increased P53 levels. Immunofluorescence analysis revealed an increase in nuclear P53 levels in IGF-1Repi−/− mice. To examine if the increased nuclear P53 is responsible for the enhanced hyperproliferation and inflammation in IGF-1Repi−/− mice, we crossed P53 floxed and IGF-1R floxed mice with the K14-Cre mice. Combined epidermal inactivation of P53 and IGF-1R−/− counteracted the prolonged epidermal hyperproliferative and inflammatory response. Together, our data indicate that loss of epidermal IGF-1R signalling controls the UVB induced hyperproliferative and inflammatory response through regulation of p53 activity. These results together with our previous results showing that IGF signalling promotes proliferative potential during development suggest that differential cues determine whether epidermal IGF-1R signalling promotes or inhibits growth. At the moment we are analysing the underlying mechanisms by which IGF regulates p53 dependent proliferation and inflammation. A better understanding of this mechanism is crucial translate our knowledge towards medical applications for diseases like skin cancer and diabetes, in which IGF signalling plays an important role.

Pruritus

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Capsaicin 8% patch for brachioradial pruritus

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Introduction: Brachioradial pruritus (BRP) is a form of neuropathic itch which is classically localized on the dorsolateral arms corresponding to the distribution of dermatome C6. BRP is associated with pathological changes of the cervical spine like spinal stenosis. Current treatment recommendations include using neuroleptics like gabapentin or pregabalin. Capsaicin 8% patch was developed to treat peripheral neuropathic pain.
Patients: We treated 16 BRP patients (3 m, 13 f; 60.5 (±7.1) years) with a capsaicin 8% patch. They had BRP for an average of 90.7 months (SD ±60.3). Previous treatment with antihistamines, neuroleptics, antidepressants or a combination of these was not completely successful.
Methods: Before and after three weeks of treatment, biopsies were taken from lesional and non-lesional skin; patients completed the Dermatology Life Quality Index questionnaire (DLQI), and the Hospital Anxiety and Depression Scale (HADS); intensity of pruritus was assessed by the visual analog scale (VAS, range 0 to 10). Capsaicin 8% patch was applied once for 60 min under highly controlled conditions and local anesthetic.
Results: All patients showed good response to treatment. The mean value of pruritus intensity was significantly reduced from 5.8 VAS points (SD ±2.3) before treatment to 1.1 VAS points (SD ±1.1) ($P < 0.001$). The mean value of pain intensity was significantly reduced from 4.2 VAS points (SD ±3.3) to 0.8 VAS points (SD ±1.0) ($P < 0.001$). DLQI score was reduced from 8.9 (SD ±7.1) to 4.3 (SD ±4.2) ($P < 0.05$). In the HADS, the score on anxiety decreased from 8.1 (SD ±3.9) to 6.1 (SD ±3.7), and the score on depression from 6.6 (SD ±3.8) to 6.3 (SD ±3.5). There was reduced intraepidermal nerve fiber density in lesional skin before therapy. The capsaicin receptor TRPV1 in lesional skin was decreased before and increased after treatment ($P < 0.005$). There were mild to moderate, usually localized and self-limited side effects.
Conclusion: The high level of response suggests that capsaicin 8% patch may present a novel, effective treatment strategy in BRP.

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Development of a prurigo activity score (PAS) for Prurigo nodularis

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Prurigo describes a scratch-related reaction pattern in chronic pruritus. Prurigo nodularis (PN) is used to designate a prurigo in which nodules are present. However, lesions in prurigo do not have a uniform appearance, but include a broad range of lesional types. Currently, no unifying terminology is available and no validated activity scores exists. The aim of our study was to develop a classification and a tool to monitor PN patients during therapy in clinical trials and daily routine. In an expert panel, we discussed and re-defined the clinical features of PN. Prurigo can be defined by (a) the clinical type of lesions (papules, nodules, plaques, ulcers), (b) activity (presence of excoriations or scars) and (c) number of lesions. We examined 136 patients with 156 prurigo lesions (143 SD; median: 107; range: 2–565). Based on observation and results of previous studies we defined a prurigo activity score (PAS), which includes besides other items a grading system (stage I–IV: active/healed prurigo lesions) and a method for measuring marker lesions. We could show that the item activity significantly correlates with pruritus intensity ($P < 0.001$, Spearman's rank correlation: $r = 0.36$) and quality of life ($P < 0.001$, $r = 0.4$). Further validation is needed and the determination of a score calculation is in progress.

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High epidermal kappa opioid receptor expression levels dominate atopic dermatitis while in prurigo nodularis mu opioid receptors are abundantly expressed

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Translational research currently aims to identify potential candidates for a target-specific therapy in pruritic dermatoses including atopic dermatitis (AD) and prurigo nodularis (PN) which is urgently needed. Potential target candidates are the peripherally expressed kappa (OPRK1) and mu (OPRM1) opioid receptor, which are expressed in various cell types in the skin, such as keratinocytes, macrophages, fibroblasts and sensory nerve fibers. The aim of the present study was to determine the cutaneous expression level of OPRK1 and OPRM1 in the lesional, pruritic skin by means of immunofluorescence, Western blot and DNA-microarray analysis. In total, 112 biopsies of patients with atopic dermatitis (AD, $n = 37$) and prurigo nodularis (PN, $n = 51$) have been analyzed and compared to healthy controls (HC, $n = 24$). The studied group consisted of men (44.6%) and women (55.4%) with a mean age of 54.8 ± 17.2 years. The OPRK1 was found in all patients mainly in the epidermal basal keratinocytes and, suprabasally in AD and PN whereas dermal inflammatory cells expressed OPRK1 only weakly. On protein level we detected the receptor via Western blot analysis on 55 kDa. The signal was significantly higher in AD ($P < 0.05$) compared to HC and no difference was seen between PN and HC skin. Concerning OPRM1, no difference was detected between HC and AD but in PN we found a 1.4-fold increase compared to HC ($P = 0.522$). To identify the localization of the OPRK1 signal precisely, we further analyzed the epidermis and dermis separately. We found that the OPRK1 is mainly expressed in the epidermis; in the dermis only a weak signal was identified in patients with AD ($P < 0.001$). Via DNA-microarray analysis we investigated the gene-expression levels of OPRK1 and OPRM1 in lesional and non-lesional skin of patients with PN, compared to HC. We found no differences between the three groups for both genes. These data support our OPRK1 Western blot results. Concerning the OPRM1, it may be speculated that translational factors seem to lead to an increase in the protein expression as found in PN. Our results indicate that peripheral OPRK1 is mainly expressed in the epidermal keratinocytes and is an interesting target especially in AD. Though PN is clinically often related to atopic dermatitis or atopic predisposition, our results favour an opposite role of opioid receptors in PN. It can be speculated that topical kappa agonists are effective in atopic dermatitis but less effective in PN. Current studies investigate the role of epidermal OPRK1 using novel peripheral agonists.

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Relationship between control of scratching, agreeableness and skin-related shame in patients with skin diseases

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Aim: It has been shown that watching an experimental video (EV)on skin diseases or crawling insects induces scratching in skin patients and healthy controls. However, during former investigations we got the impression that some participants scratched more often immediately after the video presentation – maybe because they suspected that the video induced itch and thus used mechanisms to control their scratching. This study differentiates between persons who controlled their scratching during the presentation of the EV and persons who did not and investigates whether these groups differ concerning the increase in scratching from EV to wash-out period. Moreover, the study analyzes whether the alteration in scratching from EV to wash-out period is associated with personality traits.
Methods: 41 skin patients (atopic dermatitis (AD) and psoriasis) and 31 healthy controls were presented two videos in counterbalanced order: an itch inducing EV on skin diseases or crawling insects and a neutral video (NV) on 'the skin – the communication organ'. After each video presentation a 20 min wash-out period followed. The study participants were video recorded during the video presentations and during the first 5 min of the wash-out periods. Afterwards they were divided into two groups by median split: 'uncontrolled' (increase in scratch movements from NV to EV) and 'controlled' persons (no change or decrease in scratch movements from NV to EV). The number of scratch movements that occurred during the different parts of the investigation was rated by two independent persons. In addition, participants filled in questionnaires to measure personality traits, attitude towards touching, skin-related shame and disgust.
Results: A significant time*group effect [$F(1/67) = 7.493$; $P = 0.008$; $\eta^2 = 0.101$] was revealed, which indicated that 'uncontrolled' participants showed a decrease in the number of scratch movements from EV to wash-out period, while 'controlled' participants showed an increase in the number of scratch movements from EV to wash-out period. Furthermore, in patients with skin diseases the increase in the number of scratch movements from EV to wash-out period was associated with high scores concerning agreeableness and skin-related shame ($R = 0.191$). In contrast, in healthy controls the increase in scratching from EV to wash-out period was not significantly linked to personality, attitude towards touching, skin-related shame or disgust.
Discussion: This study showed that 'controlled' and 'uncontrolled' persons differ in their alteration in scratching behavior from EV to wash-out period. Moreover, in patients with skin diseases the alteration in scratch movements from EV to wash-out period was associated with agreeableness and skin-related shame: The more agreeable the patients were and the more they felt embarrassed because of their skin, the more they controlled their scratching during the video presentation in comparison to the wash-out period. These results are in line with our previous findings, which indicated that a low increase in scratching from NV to EV was associated with being agreeable in AD-patients. Thus, being agreeable seems to hinder patients from scratching in certain situations and should therefore be

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strengthened. Furthermore, patients who strongly feel ashamed because of their skin, seem to develop mechanisms to refrain from scratching when being observed - maybe in order not to worsen their skin appearance.

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The prevalence and burden of itch among dermatological outpatients and controls in 13 European countries

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Introduction: Itch is the most common symptom in patients with dermatological diseases and many patients report itch as the most stressful part of their disease.
Objectives: a) Describing the prevalence and intensity of itch among dermatological outpatients and healthy controls in 13 European countries.
b) Describing the social and socioeconomic predictor variables of itch in the sample.
c) Giving an overview of the burden of itch and influencing factors (impact on quality of life, depression and anxiety) in the sample.
Material and methods: The design is cross-sectional. In dermatological clinics in 13 European countries, a questionnaire was filled in by 250 consecutive outpatients and 125 healthy controls. The assessment included sociodemographic background information, disease specific variables, comorbidities and standardized questionnaires to assess quality of life (Eq5D, DLQI) and anxiety and depression (Hospital Anxiety and Depression Scale). A clinical examination was performed to clarify the diagnoses by a dermatologist. Three questions were used to assess itch: an item on the presence of itch or, and if yes the duration (> < six weeks) and intensity of itch (visual analogue scale from 0 to 10). The study was approved by the ethical committee of Oslo and from each participating country.
Results: The total number of responders was 4994 (3635 patients and 1359 controls). The prevalence of itch was 54.4% in patients and 8% in controls with highest rates for prurigo (87.5%) and lowest for nevi (23.4%). The chronicity was similar in patients (72.0% chronic itch) and controls (75.3% chronic itch). The intensity (range 0–10) for patients currently suffering from itch (intensity for all patients in brackets) was highest in patients with prurigo VAS = 7.3 ± 2.3 (7.3), lowest in patients with non-melanoma skin cancer VAS = 4.0 ± 2.4 (2.8) and controls VAS = 0.5 ± 1.5 (3.6).
The occurrence of itch in patients was associated with low socioeconomic status and the presence of somatic comorbidities but not with factors like gender or age.
The patients and controls reporting itch were more depressed, more anxious, and had more limitation on all items of the EQ5D than those without itch. Chronicity had no impact on these factors. Intensity was associated with anxiety, depression and impact on quality of life for the patients but not for the controls.
Conclusions: Itch is a common symptom in patients with different skin diseases and in healthy controls. Itch occurs not only in patients with classical itch diseases like eczema or psoriasis but also in diseases like alopecia or nevi (after controlling for skin comorbidities). Chronicity and intensity of itch varies among different skin diseases. Chronicity is comparable between patients and controls.
The occurrence of itch has a great impact on anxiety, depression and the quality of life, which was similar in both groups, patients with skin diseases and controls.

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IL-31 does not induce immediate itch in atopic dermatitis patients and healthy controls after skin challenge

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Interleukin-31 (IL-31) is thought to be one of the key mediators involved in chronic pruritus in various pathologic conditions, such as atopic dermatitis (AD) or cutaneous T cell lymphoma. As of today, this feature of IL-31 was tested *in vivo* only in animal models including mice and dogs. Here, we challenged ten AD patients and 10 healthy individuals with IL-31 and NaCl (negative control) and 20 healthy controls with histamine (positive control) by skin prick testing. Itch and local inflammatory responses of the skin were assessed for up to 72 h using a visual analogue scale (VAS) for itch intensity and mexameter analysis for assessment of erythema intensity. While all of the histamine challenged subjects developed immediate pruritus (i.e. within the first 5 min), only one IL-31- and two of the NaCl-challenged subjects reported immediate itch at the provocation site (short lasting, for 2–6 min). Nine subjects (5 AD patients) reported late itch responses to IL-31 challenges with a mean delay of 143 min. No subject reported late itch responses to histamine or NaCl-testing. There was no significant difference in IL-31-induced itch start time, duration and intensity between AD patients and healthy volunteers. We therefore conclude that IL-31 does not induce immediate itch responses in humans. The late onset of IL-31 induced itch in some individuals supports the notion that IL-31 may exert its pruritic effect indirectly, for example via keratinocytes and secondary mediators, rather than through its receptors on cutaneous nerves.

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Serum autotaxin activity correlates with pruritus in paediatric cholestatic diseases

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Background: Pruritus is a common symptom of cholestatic liver disorders. The underlying mechanism of itching still remains elusive. Lysophosphatidic acid (LPA) was identified as potential pruritogen in sera of cholestatic patients. Increased levels of autotaxin (ATX), the lysophospholipase forming LPA, were specific for pruritus of cholestasis in adult patients irrespective of the underlying hepatobiliary disorder. The present study aimed at evaluating ATX and bile salt levels in children with pediatric cholestatic diseases presenting with or without itching.
Methods: A cohort of 48 children consisting of patients with Alagille-syndrome ($n = 12$), biliary atresia ($n = 2$), neonatal sclerosing cholangitis ($n = 1$), progressive familial intrahepatic cholestasis type 2 ($n = 1$), bilhemia ($n = 1$), bile salt synthesis defects without bile salt supplementation (3β-hydroxy-C27-steroid-oxidoreductase ($n = 7$) and Δ4-3-oxosteroid-5β-reductase deficiency ($n = 2$)) and healthy children ($n = 22$) were studied. Serum ATX activity and total serum bile salts (TBS) were determined enzymatically, ATX protein content was semi-quantified by western blotting. Itch intensity was quantified on a 3-point scale adapted for children.
Results: Pruritus was present in most children (14 out of 17) with pediatric cholestatic disorders, but not reported in those with bile salt-synthesis deficiencies. Serum ATX activity was increased in pruritic children with Alagille-syndrome (meanSD: 16.85.1 nmol/ml/min) compared to non-pruritic cholestatic children with bile salt synthesis defects (10.44.7 nmol/ml/min; $P < 0.01$) and healthy controls (7.62.3 nmol/ml/min; $P < 0.001$). Two additional children with Alagille-syndrome without pruritus had low ATX activity (mean: 4.6 nmol/ml/min). ATX protein levels closely correlated with serum ATX activity. In contrast to TBS or bilirubin, serum ATX activity showed a linear correlation with itch intensity ($r = 0.64$, $P < 0.001$). No correlation was observed between ATX activity and TBS or bilirubin levels. ATX mRNA expression in HepG2 cells was not induced by FXR ligands.
Conclusions: Serum ATX activity correlated with itch intensity in cholestatic children. Bile salts neither correlated with presence of pruritus nor increased ATX expression *in vitro*. ATX inhibitors may be useful antipruritic agents in paediatric cholestatic disorders.

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Further characterization of experimentally-induced non-histamine itch as a specific model for analysing topical antipruritics

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Most human *in vivo* studies on anti-pruritic efficacy of topical products use either inflammatory skin conditions or histamine as an itch-inducer. The disadvantages of these models are that standardized testing is difficult in diseased skin and that histamine-induced itch is relevant in very few causes of chronic pruritus, mainly urticaria and mastocytosis. Therefore, the aim of the study was to further characterize a non-histamine itch model induced by Cowhage spicules with non-invasive biophysical methods. Cowhage induces a histamine-independent, PAR-2-mediated itch which better reflects chronic pruritus for example in atopic dermatitis. In the second part of the study the Cowhage-induced itch was used as a specific model for testing the antipruritic efficacy of a topical polidocanol 3% formulation.
First, we assessed transepidermal water loss as a measure of barrier function, volumetry and erythema as a parameter for inflammation along with specific itch parameters (itch intensity and itch duration) in 20 healthy volunteers. Subsequently we performed a placebo-controlled, randomized trial with topical application of a Polidocanol 3% formulation in 45 volunteers in experimentally induced itch.
No barrier damage and only a slight but not significant increase of erythema values were measured after cowhage provocation. The itch characteristics (mean intensity, duration, area under the curve-AUC) were comparable for histamine- and Cowhageinduced itch. AUC for itch and maximal itch after Cowhage application in the areas pretreated with polidocanol were significantly lower than in the areas pretreated with placebo. This itch reduction by polidocanol compared to placebo was not detectable in the histamine-induced itch.
The present study showed the specific induction of non-histaminic pruritus without a significant barrier alteration or induction of inflammation. The second part of the study confirmed the validity of the itch model and showed a significant anti-pruritic effect of 3% polidocanol versus placebo without any relevant side effects in the volunteers in the Cowhage model.

Tumor Biology

P243

Strong reduction of Ago2 expression in melanoma and cellular consequences

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Processing of microRNAs (miRNAs) is a multistep and highly controlled process. Lately, deregulation of miRNA expression was observed in several types of cancer including melanoma. Commonly, this deregulated expression was attributed to chromosomal aberration or promoter regulation but changes in the miRNA processing enzymes have not been analyzed in detail until today.
In melanoma, we revealed strong reduction of Argonaute2 expression compared to primary melanocytes. Interestingly, this change was not observed in other kinds of cancer. In all melanoma cell lines and tissue samples, the reduction in Ago2 expression was only found on protein level, whereas the mRNA level stayed unchanged hinting to post-transcriptional regulation.
Changes in Ago2 expression, as a key player of the RNA-induced silencing complex (RISC), could result in modulated functionality of regulatory RNAs. This would also result in consequences in therapeutic applications of short interfering RNAs (siRNAs), short hairpin RNAs (shRNAs) and miRNAs. We could show that re-expression of Ago2 in melanoma cell lines leads to increased functionality of siRNAs and shRNAs and revealed strong improvement of regulatory effects.
In summary, we identified melanoma-specific downregulation of Ago2 protein expression resulting in reduced RNAi efficiency in melanoma cells. These findings will help to understand the molecular basis of malignant melanoma and can potentially help to improve therapeutic strategies based on si-, sh- or miRNAs.

P244

The role of SOX10 in melanoma cell invasion

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SOX10 plays a key role in the development of melanocytes from neural crest precursor cells. It activates the master regulatory gene for melanogenesis, microphthalmia-associated transcription factor (MITF), and melanogenic enzymes. Recently, a crucial function of SOX10 in melanoma initiation and melanoma cell proliferation and survival has been described. However, the distinct functions and relevant target genes of SOX10 in melanoma remain widely unknown.
Confirming previous results, we observed an increase in melanoma cell death after SOX10 inhibition within 3 to 5 days. We have demonstrated that SOX10 is not only necessary for survival, but also for melanoma cell invasion at a time-point, when cell death is not affected yet. Furthermore, blocking apoptosis and necroptosis reduced cell death induced by SOX10 inhibition, but did not restore the invasive capacity of melanoma cells. Therefore, the influence of SOX10 on melanoma cell invasion seems to be independent from its role in proliferation and survival.
Recently, we identified melanoma inhibitory activity (MIA) – a key player in melanoma cell migration – as a direct target gene of SOX10. Ectopic MIA expression partly restored the invasive capability of SOX10-inhibited melanoma cells, indicating that MIA is involved in SOX10-dependent invasion.
When investigating the effect of SOX10 overexpression in three different melanoma cell lines, increased invasion was observed in transwell matrigel assays and in a 3-D spheroid model. Strikingly, SOX10 overexpression did not enhance cell proliferation, in line with the hypothesis that SOX10 affects melanoma cell invasion independently from cell viability. We have previously demonstrated that SOX10 and MIA expression levels strongly correlate in melanoma cell lines. Interestingly, SOX10 overexpression increased MIA levels in MIA-positive, but not in MIA-negative cells. Therefore, SOX10 expression can promote invasion without upregulation of MIA and likely regulates further genes that have an important function in melanoma cell invasion. Microarray analyses of SOX10-inhibited and -overexpressing melanoma cells will help to identify further target genes that mediate the influence of SOX10 on cell invasion.
In summary, our data provide evidence for a critical role of SOX10 in melanoma cell invasion and highlight its role as a therapeutic target in melanoma.

P245 (O01)

Alpha-melanocyte-stimulating hormone reduces skin carcinogenesis by inhibiting the expansion of myeloid-derived suppressor cells

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The neuropeptide alpha-melanocyte-stimulating hormone (α-MSH) is a potent immunomodulator and was shown to induce tolerance. Accordingly, in mouse models of skin inflammation, α-MSH up-regulated the numbers and suppressive activity of regulatory T cells (Treg). Since the development of skin cancer is controlled by the immune system and Treg are known as suppressors of anti-tumoral immunity we investigated the effects of α-MSH on skin carcinogenesis. Surprisingly, in a two-stage chemo-carcinogenesis study α-MSH-treated mice developed significantly fewer skin tumors compared to controls although these mice exhibited systemically increased numbers of functional Treg after DMBA/TPA treatment. Moreover, as demonstrated by immunohistology, tumors of PBS-treated controls showed a high degree of dysplasia whereas tumors of α-MSH-treated mice presented as small papillomas with less invasive cells and a reduced proliferation of abnormal keratinocytes. Interestingly, the total numbers of CD8+ T cells were significantly increased in tumor tissue and tumor-draining lymph nodes from α-MSH-treated mice compared to controls. These CD8+ T cells expressed higher levels of cytotoxic markers like granzyme B, Fas ligand, IFN-γ, perforin, the activating CD94 receptor (NKG2B/D) as well as the transcription factors Runx3 and Eomes, both associated with cytotoxic function. In addition, *in vitro* cytotoxicity assays revealed an increased tumor-specific cytolytic activity of CD8+ T cells from α-MSH-treated mice versus PBS-treated controls. Of note, this effect was clearly mediated by α-MSH since treatment of mice with KdPT, a tripeptide related to the C-terminus of α-MSH and known to mediate similar immunosuppressive effects, did neither reduce skin carcinogenesis nor up-regulate the numbers of cytotoxic CD8+ T cells (CTL). Immunomodulatory effects of α-MSH are mainly mediated by binding to the melanocortin-1 receptor (MC-1R). Hence, we analyzed whether signaling through MC-1R might be required for the expansion of CTL and the reduced skin carcinogenesis. Notably, α-MSH treatment of C57BL/6Je/e mice lacking a functional MC-1R did neither reduce tumor development nor up-regulate CTL function indicating a central role of MC-1R signaling during the α-MSH-mediated induction of MHC class I-restricted anti-tumoral immunity. Next, we analyzed the underlying cellular mechanism in more detail and surprisingly, detected reduced levels of myeloid-derived suppressor cells (MCSC) in bone marrow and peripheral blood from α-MSH-treated mice compared to PBS-treated controls. Moreover, compared to controls MDSC from α-MSH-treated mice were less efficient in suppressing the proliferation of tumor-specific CD8+ T cells suggesting that α-MSH up-regulated MHC class I-mediated anti-tumoral immunity by preventing the generation of functional MDSC. In support of this, α-MSH impaired the *in vitro* generation of MDSC from bone marrow by interfering with NF-κB signaling. To analyze whether the effect of α-MSH on MDSC function and CTL expansion was restricted to the inflammatory tumor environment in DMBA/TPA-induced carcinogenesis, Grm1EPv mice, which spontaneously develop melanomas, were injected with α-MSH or PBS. Although α-MSH slightly reduced MDSC numbers, the tumor incidence as well as the CTL function was similar in both groups. Together, our data demonstrate that in inflammation-mediated skin cancer α-MSH, by binding to MC-1R, prevents the induction of suppressive MDSC resulting in the up-regulation of MHC class I-restricted anti-tumoral immunity and reduced skin carcinogenesis, thus suggesting α-MSH as potential therapeutic option for inflammation-mediated cancer.

P246

The PI3K inhibitor BKM120 has potent antitumor activity in melanoma brain metastases *in vitro* and *in vivo*

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In melanoma, the RAF-MEK-ERK and PI3K-AKT-mTOR signaling pathways play a major role in melanoma progression and drug resistance. On the basis of significant improvement in overall survival, the BRAF inhibitor vemurafenib gained FDA approval for the treatment of patients with metastatic BRAFV600E mutated melanoma. However, vemurafenib appears to be less effective in melanoma brain metastases, and brain metastases are the most common cause of death in patients with metastatic melanoma. In our previous study we reported that matched brain and extracerebral metastases from melanoma patients had identical ERK, p-ERK, and AKT immunohistochemistry staining patterns, but there was hyperactivation of the AKT survival pathway in the brain metastases. Mutational analysis revealed no differences in BRAF, NRAS, or KIT mutation status in matched brain and extracerebral metastases, indicating that hyperactivation of AKT in melanoma brain metastases does not depend on the mutation status of these genes. The current study aims to investigate the mechanisms of AKT hyperactivation and the antitumor activity of the PI3K inhibitor BKM120 in melanoma brain metastases *in vitro* and *in vivo*.

To simulate the tumor environment of brain metastases and extracerebral metastases, brain and matched extracerebral metastatic melanoma cells were stimulated by astrocyte- and fibroblast-conditioned medium, respectively. Both brain and extracerebral metastatic melanoma cells stimulated by astrocyte-conditioned medium showed higher AKT activation and invasiveness in a transwell matrigel invasion assay than cells stimulated by fibroblast-conditioned medium. The PI3K inhibitor BKM120 inhibited the phosphorylation of AKT and the growth of >10 cell lines achieving growth inhibition rates of up to 80%. These effects did not depend on BRAF, NRAS or KIT mutation status. Furthermore, BKM120 potently induced apoptosis in melanoma cells and significantly inhibited the tumor growth of human brain metastatic melanoma cells in the brain of nude mice as shown by MRI scans.

Brain-derived factors induce hyperactivation of the AKT survival pathway and promote invasiveness and drug resistance of melanoma cells in the brain. The PI3K inhibitor BKM120 inhibits activation of the AKT survival pathway and demonstrates potent antitumor activity in melanoma brain metastases *in vitro* and *in vivo*.

P247 (O24)

Tumor-suppressive function of ASC dominates over inflammasome activation in human squamous cell carcinoma

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In a mouse-model of inflammation-induced cutaneous squamous cell carcinoma (SCC), we recently demonstrated that IL-1-signaling and inflammasome activation promotes cancer development. While IL-1R1−/− and caspase-1−/− mice showed a lower tumor incidence, suggesting a role of the inflammasome in carcinogenesis, mice deficient for the inflammasome adaptor ASC were not protected against cancer development. Using promoter-specific conditional ko mice, cell type-specific functions of ASC as a tumor promoter in myeloid cells, but as a tumor suppressor in epithelial cells were demonstrated. To dissect these opposing roles in human skin cancer, we now investigate human SCC cell lines, primary human keratinocytes and primary SCCs of various differentiation stage, tumor-thickness and outcome to determine whether ASC expression correlates to the progression or metastasis of the tumor.

Several SCC cell lines and primary keratinocytes were checked for ASC expression by real time PCR. Interestingly, these cell lines that grew more rapidly showed decreased ASC expression. Using methylation specific PCR, the mechanism how ASC is silenced was identified. Lost ASC expression could be reversed by treatment with the demethylating agent 5-aza-2′-deoxycytidine. As ASC not only regulates proliferation but is also needed for IL-1 activation via inflammasomes, we investigated the influence of ASC expression and 5-aza-2′-deoxycytidine on caspase-1 activation in keratinocytes. Upon treatment with UVB, a known activator of the inflammasome in keratinocytes, cleaved caspase-1 was only detected in the supernatants of cell lines expressing ASC. However, after treatment with the demethylating agent, not only ASC, but also caspase-1 cleavage was restored. Methylation induced silencing of ASC can therefore influence proliferation, but also IL-1/caspase-1 activation in keratinocytes. We then analyzed primary human SCC samples for the expression of ASC by immunohistochemistry to evaluate the biological relevance of ASC. Here we correlated clinical outcome (metastasis vs. no metastasis), tumor thickness and histological differentiation. While ASC expression in the 33 specimen did not correlate with clinical outcome or tumor thickness, tumors with higher dedifferentiation (G3) more frequently silenced ASC than G1 tumors.

These data suggest that the tumor-suppressive capacity of ASC dominates in the tumor cells. Intrinsic functions, such as differentiation or proliferation do not demand activated caspase-1, but depend on the non-inflammasome functions of ASC. In contrast to myeloid cells, in epithelial tumor cells impaired caspase-1- and IL-1beta-activation does not inhibit tumor growth.

P248

Role of PKCbeta in melanoma biology

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The molecular basis for melanoma development and progression has been investigated in recent years in a series of large-scale genetic and genomic studies. However, functional analyses of many of the candidate genes found in these studies are still missing. In a recently performed RNAi loss-of-function screen, we identified a series of genes active in melanoma and relevant for melanoma cell growth and survival. One of our top candidates was PKCbeta. PKCbeta is a signalling kinase that phosphorylates and activates tyrosinase and is bound to the melanosome by the receptor for activated C-kinase-I (RACK-I). PKCbeta knockdown had a strong impact on melanoma cell proliferation, clonogenicity and migratory capacity *in vitro*, and significantly reduced lung colonisation of stably transduced melanoma cells in mice. Moreover, in humans expression of PKCbeta differed in benign nevi, primary melanomas, and distant cutaneous and other organ metastases. PKCbeta expression was significantly higher in primary melanomas and metastases as compared with benign melanocytic nevi. These results suggested that PKCbeta might act as an oncogene during melanoma initiation and progression and therefore might be a therapeutic target. Indeed, treatment of melanoma cells with PKCbeta-specific inhibitor enzastaurin significantly reduced melanoma cell growth, but had only moderate effects on benign fibroblasts. Enzastaurin treatment induced expression of pro-apoptotic molecules p53, p21 and Bax in melanoma cells. Moreover, cell cycle analysis showed a concentration-dependent increase in G1-phase arrest of melanoma cells upon PKCbeta inhibition and apoptosis induction. Taken together, PKCbeta seems to play an important role in melanoma biology and may be targeted by small molecule inhibitor enzastaurin.

P249

The role of polarity proteins in melanocyte homeostasis and melanoma

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Cell polarization is crucial during development and tissue homeostasis and is regulated by conserved proteins of the Scribble, Crumbs, and Par complexes. The partitioning defective (Par) complex consists of the proteins Par3, Par6 and aPKC. We recently revealed a dual function of Par3 in a Ras-driven skin tumorigenesis model: epidermal Par3 deletion resulted in strongly reduced numbers and growth of papillomas, unraveling pro-oncogenic functions of Par3. Loss of Par3, however, also predisposes mice to the formation of another epidermal tumor type, called keratoacanthoma, highlighting also tumor-suppressive functions of Par3 depending on the cellular context (Iden et al., 2012).

Interestingly, epidermal Par3 deletion also resulted in melanocytic hyperplasia and increased melanoma formation, suggesting that epidermal polarity proteins are important for melanocyte homeostasis.

Here we investigate how melanocyte homeostasis is regulated by neighboring keratinocytes and how loss of the polarity protein Par3 in the epidermal layers impact the development of melanoma, a frequent non-epithelial skin cancer characterized by high lethality and poor treatment options. By combination of *in vitro* co-cultures and *in vivo* analyses of normal and tumor tissue, we currently investigate the role of these conserved regulators of cellular asymmetry in mice and human. Our initial data indeed revealed increased melanocyte numbers and altered cell morphology when directly co-cultured with Par3 KO keratinocytes as compared to control keratinocytes indicating a non-cell autonomous role of Par3 in the regulation of melanocyte features. To assess potential intrinsic roles of Par3 in melanocytes and melanoma, we investigated polarity protein expression and found a robust expression of all members of the Par3 complex in melanocytes, melanoma cells and melanoma. Interestingly, localization studies revealed that Par3 localizes to sites of intercellular adhesion in both, melanocytes and melanoma cells. Importantly, RNAi-mediated Par3 depletion in melanoma cells affects cell proliferation, morphology and the localization of cell-cell contact proteins, indicating a critical role of melanocyte-expressed Par3 in melanoma growth and progression.

Taken together, our initial data indicate that polarity proteins control both the homeostasis and oncogenic fate of various cell populations in the skin. Future analyses will address the functional role of polarity proteins in melanoma, since alterations in cellular morphology, differentiation programs as well as increased migratory potential are key processes underlying melanoma progression and metastasis rate. We thereby expect to reveal novel molecular mechanisms that are important in understanding a highly relevant cancer and ultimately unraveling novel molecular targets for the development of more efficient therapies.

P250 (O33)

ADAM-9 deletion in an animal model with spontaneous melanoma development reduces lung metastases formation

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ADAM-9 is a proteolytic and adhesive protein that belongs to the adamalysins family of proteolytic enzymes. Increased expression of ADAM-9 has been shown in several cancers including pancreatic carcinoma, breast cancer and in melanoma. In human melanoma ADAM-9 expression is localized in the areas of the tumor invading the dermis, in particular in tumor cells and adjacent fibroblastic cells. Recent studies of our group indicated that deletion of ADAM-9 in stromal cells increases melanoma growth. However, it is yet unclear what is the function of ADAM-9 expression in melanoma cells. To address this question we crossed Adam-9−/− with Hgf/Cdk4 double mutant mice, in which melanomas resembling the human histomorphology can be induced. The generated animals, which are deficient for ADAM-9 in melanoma cells as well as in stromal cells, were treated with DMBA and tumor formation was followed over time. This analysis surprisingly revealed that in the absence of ADAM-9 initially (week 4) a higher number of tumors developed, whereas at later time points (week 13) the number of developed tumors was reduced compared to control animals. Analysis of proliferation, apoptosis and inflammation of the tumors indicated that an altered proliferation of the tumor cells might be responsible for the observed differences in tumor number and size.

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Strikingly deletion of ADAM-9 resulted in a significantly reduced lung metastases formation. This effect might be attributed to decreased extravasation due to reduced adhesion of ADAM-9 deficient melanoma cells to the endothelium. This hypothesis was supported by the fact that *in vitro* ADAM-9 silenced melanoma cells exhibited a significantly reduced adhesion and transmigration through an activated endothelial cell monolayer. Taken together, these data show that ADAM-9 expressed by melanoma cells influences melanoma cell proliferation and is a pro-metastatic protein *in vivo*.

P251

Tyrosinase-related protein 2 (TRP2) is not required for p53 regulation in melanoma

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p53 is a central tumor suppressor protein and its inhibition is believed to be a prerequisite for cancer development. In approximately 50% of all malignancies this is achieved by inactivating mutations in the p53 gene. However, in several cancer entities, including melanoma, p53 mutations are rare. It has been recently proposed that tyrosinase-related protein 2 (TRP2), a protein involved in melanin synthesis, may act as suppressor of the p53 pathway in melanoma. To scrutinize this notion we analysed p53 and TRP2 expression by immunohistochemistry in 172 melanoma tissues and did not find any correlation. Furthermore, we applied 3 different TRP2 shRNAs to 5 melanoma cell lines and could not observe a target-specific effect of the TRP2 knockdown neither on p53 expression nor on p53-dependent reporter gene activity. Likewise, ectopic expression of TRP2 in a TRP2-negative melanoma cell line had no impact on p53 expression. In conclusion, our data suggest that p53 repression critically controlled by TRP2 is not a general event in melanoma.

P252

Chronic activation of keratinocytes by the peptide peptidase like 3 (SPPL3): a crucial factor in melanoma pathogenesis?

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Melanoma arises through multiple genetic mutations critical for survival and proliferation. In addition the tumor microenvironment can play an important role in influencing melanoma progression. Here we present data indicating an involvement of keratinocytes in the early steps of melanocytic transformation.
Our date propose the model of 'wrong' protease cargo from melanoma cells to keratinocytes leading to an altered protein expression pattern (PBC, protein bar code). Here we analyzed tissue sections of all steps of melanoma development (from benign nevi to invasive melanoma) using the MELC-technology. The MELC (Multi Epitope Ligand Cartography)-technology is a cyclical immunofluorescence imaging technology to investigate up to hundred different proteins in one tissue/cell sample simultaneously for their topographical distribution. In order to determine a detailed PBC of melanoma cells, we screened approximately 950 antibodies for a melanoma-specific staining. This large-scale screening process resulted in a defined antibody library to characterize all types of common nevi and melanoma. Surprisingly we detected a highly modified PBC of keratinocytes during the transformation from melanocytes to melanoma cells. These melanoma-associated keratinocytes showed in more than 20 proteins an altered protein expression. Interestingly the changes in deregulated protein levels are already noticeable at the stage of the dysplastic nevus, a precancerous mole. In order to simulate the tissue situation *in vitro* we cocultured keratinocytes with primary melanoma cells. Colocalization maps revealed a shuttling of SPPL3 via the melanocytic dendrites into the keratinocytes. The translocated SPPL3, a member of the gamma secretase family, induced the expression of several factors like PPAR gamma and TAp73. MELC-analysis of the *in vitro* keratinocytes identified an almost identical change in the PBC to the one detected in tissue. SPPL3 activity is closely linked to the activation of ADAM10, one of the most critical factors in melanoma development (Lee et al., 2013). The inactive mutant of SPPL3 was able to block while wild-type SPPL3 enhanced ADAM10 activation in HEK 293T cells.
Our study suggests that activated proteases are shuttled from melanoma cells into keratinocytes. These 'wrongly' shuttled proteases induce the activation of signaling pathways and expression of new factors. This altered signaling in melanoma-associated keratinocytes showed a high similarity of the pathways known for being deregulated in melanoma cells. Melanoma-associated keratinocytes appear to be a highly sensitive mirror in the early steps of melanoma development. Furthermore the PBC of keratinocytes may be used in histological melanoma diagnostics.

P253

miR-638 promotes melanoma metastasis and is repressed by transcription factor AP-2alpha

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In a recent miRNA profiling study on melanoma, miR-638 was found to be overexpressed in metastatic stages. *In vitro*, depletion of endogenous miR-638 in melanoma cells induced significant levels of apoptosis mediated by the p53 pathway. In contrast, overexpression of miR-638 enhanced proliferation, clonogenicity, migration and anchorage-independent growth of melanoma cells. Finally, in a mouse xenograft model, human melanoma cells stably overexpressing miR-638 exhibited a significantly higher potential for lung colonization as compared with non-targeting control expressing cells. Overall these results supported the oncogenic role of miR-638 in melanoma. Further analysis revealed tumour suppressors like TP53INP2 and BTG2 to be direct targets for miR-638. Interestingly, knockdown of TP53INP2 in melanoma cells significantly enhanced secretion of tumour-promoting cytokines IL-6 and IL-8. This suggested that miR-638 promotes tumourigenesis, at least in part by repressing TP53INP2 and induction of cytokines IL-6 and IL-8. Using in silico transcription factor analysis for the miR-638 gene, AP-2alpha (TFAP2A gene) was identified. AP-2alpha is a known tumour suppressor in melanoma and may also be a transcriptional repressor of miR-638. Interestingly, the majority of AP-2alpha binding sites in miR-638 promoter were located in methylation-prone CpG-rich regions. Using DNA de-methylation and chromatin immunoprecipitation (ChIP) experiments in melanoma cells, it was demonstrated that AP-2alpha-mediated repression of miR-638 is dependent on the methylation status of miR-638 promoter. Furthermore, TFAP2A expression levels were significantly suppressed by miR-638, indicating that TFAP2A is a target for miR-638. These findings provided strong evidence that TFAP2A and miR-638 regulate each other through a double-negative feedback mechanism. Taken together, it was shown that miR-638 promotes metastatic properties of melanoma cells by repressing important tumour suppressor genes like TP53INP2 and BTG2. Knockdown of miR-638 in melanoma cells induces apoptosis mediated through p53 and its target genes. AP-2alpha and miR-638 were found to regulate each other in a double negative feedback loop which may program melanoma cells in a permanent proliferative stage. Interference with this feedback loop might help to overcome treatment resistance of metastatic melanoma in the future.

P254

A 'modular' murine melanoma model to study determinants of multimodal immunotherapeutic regimens

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Metastatic malignant melanoma is a highly aggressive and chemoresistant skin cancer with poor clinical outcome. Significant breakthroughs in the treatment of this devastating disease have been made in the recent years. Small molecule inhibitors against the mutated BRAF or MEK kinases and antibody-mediate blockade of negative immune checkpoint molecules like CTLA-4 and PD1/PD-L1 prolong overall survival rates, but acquired resistance to these targeted therapies is the major obstacle for long-term remissions despite profound initial responses. Hence, malignant melanoma is a paradigm disease to exploit the synergisms of combined tumor biological and tumor immunological multimodal treatment approaches to achieve durable responses. Recent studies including ours identified diverse mechanisms of resistance to signaling inhibitors and immunotherapies in melanoma. We found that phenotypic plasticity of melanoma cells caused by a proinflammatory tumor microenvironment represents a critical route to resist a targeted T-cell immunotherapy directed against melanocytic antigens through reversible dedifferentiation without the need for acquired secondary hardwired genetic aberrations (Landsberg, …, Hölzel, Tüting. Nature 2012). Therefore we conceptualized scenarios how genetic and non-genetic sources of tumor heterogeneity could account for therapy resistance in part through reciprocal interactions (Hölzel, Bovier and Tüting. Nat Rev Cancer 2013). To address this emerging field in therapy resistance we exploited MET tyrosine kinase oncogene addiction in our HgfxCdk4R24C murine melanoma model and implemented CRISPR/Cas9 genome editing to establish a rapid pipeline to probe clinically relevant determinants of responsiveness to multimodal immunotherapeutic regimens. Preliminary results will be presented.

P255

The intracellular domain of Notch4 suppresses Twist1 and Slug through activation of Hey-1 and Hey-2

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Notch signaling exerts both oncogenic or tumor suppressive effects in cancer depending on the cellular context. Notch4 recently has been reported to be expressed in melanoma cells and to regulate the embryonic morphogen Nodal (Hardy et al., 2010). Epithelial-mesenchymal transition regulators (EMTRs) such as Twist1 and Slug are crucial for E-cadherin suppression, eventually promoting tumor progression, invasion and metastasis. We hypothesized that Notch4 might be involved in regulation of EMTRs investigating on a potential crosstalk between Notch signaling and the transcriptional activation of Twist1 and Slug. Immunoblotting and real-time PCR of different melanoma cell lines (WM35, WM9, WM164) transduced with lentiviruses encoding the intracellular domain of Notch4 (N4ICD) showed a significant decrease of Twist1 and Slug expression at protein and mRNA levels. Accordingly, up-regulation of Twist1 and Slug was observed both at protein and transcript levels in melanoma cells transfected with small interfering RNAs (siRNA) against Notch4 but not scrambled control. Canonical Notch signaling activates target genes such as Hey-1/-2, basic helix-loop-helix transcription factors, known to be effectors of Notch signaling. Probing for Hey-1and Hey-2 expression, we observed significant increased protein and transcript expression levels for both in N4ICD transduced cells. In a search for a possible effect of Hey-1/-2 on Twist1 and Slug regulation, selective knockdown of Hey-1 by siRNAs led to upregulation of both Twist1 and Slug in melanoma cells at mRNA and protein levels. Hey-2 silencing resulted in substantial increased expression of Slug but not Twist1 levels. Transfection with Hey-1 cDNAs resulted in downregulation of Slug and Twist1 in these cells. Hey-1/-2 binds to E-boxes for transcriptional regulation and we identified E-boxes on both Twist1 and Slug. Electrophoretic mobility shift assays (EMSA) demonstrated binding of Hey-1 and Hey-2 to E-boxes upstream of the transcription start sites (TSS) on both Twist1 and Slug promoters. Our data suggest that Notch4 signaling is indirectly involved in suppression of Twist1 and Slug through activation of Hey-1 and Hey-2. Loss of Notch4 might thus lead to unbalanced leverage of a suppressive threshold on EMTRs like Twist1 and Slug promoting melanoma progression.

P256

LYVE-1, a tumor- associated macrophages marker in mouse and human and its role in cancer biology

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Tumor-associated macrophages (TAMs) represent M2-like macrophages involved in tumor initiation, tumor progression and metastasis. We have previously identified the lymphatic endothelial cell marker Lyve-1 to be expressed by tumor-associated macrophages. These Lyve-1+ macrophages have been shown to participate in lymphangiogenetic processes in the cornea.
In this study we found Lyve-1 to be induced in human peripheral blood monocytes (pBMs) *in vitro* by a combined stimulation with M-CSF, dexamethasone and IL-4. In addition, Lyve-1 expression in pBMs was also achieved by co-culturing human pBMs with WM115 melanoma cells. WM115 cells were originally derived from a primary melanoma, while the WM266.4 cell line originated from a metastasis of the same patient. Co-cultivation of WM266.4 cells with pBMs did however not induce Lyve-1 expression *in vitro*. To verify the *in vitro* findings in an *in vivo* setting, different pathological stages of melanoma were immunohistochemically stained with Lyve-1 antibody. Lyve-1 was thereby expressed by a subpopulation of TAMs in all tumor stages. In order to assess, whether Lyve-1 has a tumor promoting function, the lewis lung carcinoma cell line LLC, which has been shown to be strongly infiltrated with Lyve-1 positive macrophages was injected in the right flank of Lyve-1 knockout and control mice. After 14 days of tumor growth, the tumors showed no difference in in final tumor end weight. Immuohistochemical analysis however revealed less caspase3 positive necrotic areas in LLC carcinomas of Lyve-1 knockout mice. This finding did not correlate with a difference in vessel or macrophage density.
These results suggest, that the presence of Lyve-1 might enhance the susceptibility of tumor cells for apoptotic or necrotic stimuli. Further studies are needed to better understand this effect of Lyve-1 for the tumor biology.
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P257

Characterization of TAMP1 expression and function in tumor-associated macrophages

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Tumor-associated macrophages (TAM) are important regulatory immune cells in the tumor microenvironment whose involvement in smoldering inflammatory processes in cancer and cancer metastasis have been recognized.

By gene expression profiling of bone-marrow derived macrophages stimulated with tumor conditioned medium, we have identified the novel type one transmembrane protein TAMP1 selectively expressed by TAM in colorectal carcinoma and malignant melanoma. TAMP1 is a member of a protein family involved in the regulation of the adaptive immune system. To further investigate TAMP1 *in vivo* and *in vitro*, a monoclonal antibody was generated against a n-terminal peptide sequence of the protein in rats. In order to verify the specificity of the antibody, a transgenic TAMP1 expressing monocytes-like RAW264.7 cell line was generated. These transgenic cells were analyzed by western blot and immunohistochemistry stainings. In western blot analysis two specific bands were identified by the monoclonal antibody, which points to a posttranscriptional modification of the protein. Furthermore, immunohistochemical stainings revealed a strong TAMP1 expression in TAM of both subcutaneous B16F10 melanoma and of CT26 colon carcinoma transplant tumors. To study the function of TAMP1, proliferation and apoptosis were tested in the Raw264.7 transgenic cell line but no differences were observed. Additionally, the migration was analyzed by a wound healing assay: TAMP1 positive RAW264.7 cells displayed a delayed closure of the artificial scratch. Concluding, these results indicate a possible role of TAMP1 for macrophage migration or adhesion, but further studies are needed.

P258

In melanoma, YAP1 signaling is affected by copy number alterations and its overexpression impairs patient survival

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Melanoma is a highly aggressive neoplasm that metastasizes early during progression. The genetic basis of melanoma invasion and metastasis is only partially understood. Recently it was shown that Yes-associated protein 1 (YAP1), an oncogenic driver negatively regulated by the Hippo signaling pathway, contributes to melanoma invasion. Here we show focused amplifications of YAP1, the upstream kinase PAK1, and focused deletions of its negative regulators NF2 and LATS1 in 34.5% of melanomas. YAP1 protein is highly expressed in 56% of thick ($\geq$2 mm) primary melanomas and its expression is correlated with YAP1 amplification and tumor thickness. Survival analysis of 380 primary melanomas reveals that high YAP1 expression significantly correlates with poor patient survival ($P = 0.013$). In conclusion, these results demonstrate that YAP1/Hippo signaling is a frequent target of copy number alterations and that YAP1 overexpression negatively affects survival in human melanoma.

P259

Novel expression of relaxin and its receptors in melanocytic tissues and their role for melanoma cell invasion

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The hormone relaxin enables the birth process via relaxation of smooth muscle cells in the cervix uteri and the induction of matrixmetalloproteinases in the symphysis pubica. Recently, it was shown that relaxin is up-regulated in breast, endometrium, colon and prostate cancer, where it drives invasion of cancer cells.

Here, we report the novel expression of relaxin and its receptors RXFP1 and RXFP2 in melanocytic tissues and analyze a possible role for melanoma cell invasion.

Western blot analyses demonstrated that relaxin, RXFP1 and RXFP2 were expressed in 10 different metastatic melanoma cell lines. Immunohistochemistry of melanocytic tissues (nevi: $n = 16$, primary melanomas: $n = 17$, melanoma metastases: $n = 9$) revealed a low basal expression of relaxin, RXFP1 and RXFP2 in skin and nevi, and a more prominent, patchy distribution in distinguished nests of melanoma cells in primary melanomas and metastases. Interestingly, the expression of relaxin was most prominent in the infiltrating parts of thick primary nodular melanomas at the level of the 'invasive front'. Western blot analyses using lysates of clinical samples of skin, nevi, primary melanomas and subcutaneous or lymph node melanoma metastases confirmed the increased expression of relaxin in primary melanomas and metastases.

We down-regulated relaxin in the metastatic SKMEL28 and BLM melanoma cells (with strong endogenous expression of relaxin) via siRNA, and analyzed the effect on proliferation, migration and invasion (*in vitro* in Boyden chamber assays and skin reconstructs, and *in vivo* in the rhombencephalon of the chick embryo). In a second approach, we stimulated radial growth phase SBCL2 melanoma cells (with little endogenous expression of relaxin) using recombinant human relaxin, and analyzed the effects on proliferation, migration and invasion as above. Since the experiments are still ongoing, the results will presented at the ADF in 2014.

Our data demonstrate the novel expression of relaxin and its receptors in melanocytic tissues and suggest that relaxin is involved in invasion of melanoma cells and thus in the progression of melanoma.

P260

Isoform-specific function of CEACAM1 during tumorigenesis: CEACAM1-3S, a novel prognostic biomarker in malignant melanoma

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Malignant melanoma is the most aggressive type of skin cancer characterized by continuously rising incidence and high metastatic potential. Metastatic spread is the leading course of death and cell adhesion molecules have been shown to play a major role in this process by deregulation of cell adhesive function. In tumor tissue, the expression of the carcinoembryonic antigen (CEA)-related cell adhesion molecule 1 (CEACAM1) is very dynamic. Down-regulation of CEACAM1 expression has been described for many solid tumor entities such as colorectal and breast cancer. In contrast, increased CEACAM1 expression has been detected during progression from benign skin tumors to malignant melanoma. Additionally, soluble CEACAM1 level correlates with poor patient survival.

In principle, 12 different human CEACAM1 isoforms are known which differ in their number of extracellular domains (3/4) and length of their cytoplasmic tail (S/L). However, only CEACAM1-4L, CEACAM1-4S, CEACAM1-3L and CEACAM1-3S were shown to be expressed on mRNA level in different tissues and tumor entities but their biological functions have not been analyzed so far. First we could show that all splice varinats of CEACAM1 are expressed in melanoma biopsies and, in contrast to melanocytes, in melanoma cell lines established from patient metastases.

Extensive expression analysis revealed a neo-expression of CEACAM1-3S in late stage (stage III/IV, AJCC criteria) melanoma. In addition, we could show that CEACAM1-3S expression level significantly correlates with clinical stage and patients overall survival.

To investigate the isoform-specific function of CEACAM1 we transfected the different variants in a CEACAM1-negative melanoma cell line. Real-time monitoring by using the xCELLigence System revealed the inhibitory potential of CEACAM1-3S on the process of cell migration and invasion, whereas all other CEACAM1 isoforms enhanced these cellular characteristics.

Furthermore, we found that CEACAM1 splice variants were capable to influence the immunogenicity of melanoma cells specifically by regulating the surface expression of NKG2D ligands. Strikingly, CEACAM1-3S transfected melanoma cells showed increased cell surface expression of MICA and ULBP2. Contrarily, CEACAM1-4L transfectants expressed less NKG2D ligands on their cell surface, which we could show is due to enhanced shedding of both ligands.

This is the first study identifying the impact of isoform-specific CEACAM1 expression for the disease of malignant melanoma. In addition, our study revealed the impact and importance of CEACAM1 extracellular domains for the modulation of biological functions which disproves the dogma that only the cytoplasmic tail has the potential to direct downstream signaling.

Taken together we showed that the expression of CEACAM1-3S in melanoma cells refer to a better survival rate of patients by increasing the tumor cell immunogenicity and reducing cell invasiveness. Hence, CEACAM1-3S represents a novel prognostic biomarker for malignant melanoma.

P261 (O16)

cFLIP isoforms differentially regulate CD95L-mediated cell death and Ripoptosome formation in melanoma

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Apoptosis and necroptosis are tightly controlled and regulated by an intracellular signalling platforms named Ripoptosome. The Ripoptosome is a complex containing RIP1, FADD, Caspase-8 and cFLIP and is negatively regulated by the inhibitor of apoptosis proteins (IAP's). Suppression of cIAPs either by IAP antagonist-mediated degradation or genotoxic stress leads to accumulation of RIP1 that supports Ripoptosome formation. Based on stoichiometry and activity of Ripoptosome components, it regulates cell survival, necroptosis and apoptosis. While caspase activity within the Ripoptosome promotes apoptosis, dominant RIP1 activity promotes necroptotic cell death in a RIP3 kinase and MLKL dependent manner. Different isoforms of cFLIP play a pivotal role for necroptotic Ripoptosome responses. Since the Ripoptosome exclusively forms in transformed cells, at least in keratinocyte skin cancer, this complex is a potential target in skin cancer treatment. For malignant melanoma, the relevance of the Ripoptosome or its different molecular components is currently unknown.

To investigate the role of IAP's and cFLIP isoforms for Ripoptosome formation and cell death regulation, we analysed endogenous expression of different IAP's (cIAP1/2, XIAP, survivin) and cFLIP isoforms (cFLIPL and cFLIPS) in melanoma cell lines representing different tumor stages. Both, cIAP1 and XIAP are strongly expressed in the majority of melanomas while cIAP2, survivin and cFLIP isoforms are heterogeneously expressed. To gain further insight in the role of IAP's for cell death regulation in melanomas, we analyzed sensitivity to TRAIL- or CD95L-induced cell death in the presence of IAP antagonists. Degradation of cIAPs and inactivation of XIAP activity sensitized a death ligand resistant cell line (IGR cells), or increased the sensitivity to CD95L or TRAIL-induced cell death (SK-Mel, RPM-EP, A375). These findings indicate that IAP's are critical negative regulators of CD95L- and TRAIL-induced cell death in melanoma. To investigate if the observed cell death is caspase or RIP1 kinase dependent, cell death induction was studied in the presence of the pan caspase inhibitor zVAD-fmk or the RIP1 kinase inhibitor Necrostatin-1. While death ligand (DL)-mediated cell death under control conditions was strictly caspase-dependent, cotreatment with IAP antagonist/CD95L was not fully caspase-dependent (A375 cells), indicative of caspase independent necroptosis. If note suppression of RIP1 kinase activity or knockdown of RIP1 did not protect A375 cells from caspase independent cell death, raising the possibility that other molecules are involved in this cell death response (e.g. RIP3 or MLKL). Further analysis with inhibitors of RIP3 kinase or MLKL (Necrosulfonamide) identified a RIP3 kinase and in some cell models also MLKL dependent cell death response indicative of the indispensible role of both proteins for necroptotic cell death.

To get further insight in the role of cFLIP in this context in melanomas, we analyzed the sensitivity to CD95L-induced cell death in the presence of IAP antagonists in RPM-EP melanoma cells overexpressing cFLIP isoforms. Both cFLIP isoforms protected RPM-EP cells to DL-induced cell death. However only cFLIPL, but not cFLIPS protected melanoma cells from necroptosis.

Our data demonstrate that both, IAPs and cFLIP proteins are important negative regulators of the intracellular cell death machinery in melanoma. Necroptosis induction is strongly associated with formation of the Ripoptosome. We suggest that the Ripoptosome is an important signalling platform in melanoma and may be a potential target of novel melanoma treatment.

P262

Characterization of functional domains in the Merkel cell polyoma virus Large T antigen

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Merkel cell carcinoma (MCC) is an aggressive skin cancer with viral etiology. Indeed, the Merkel cell polyomavirus (MCPyV) is found monoclonally integrated in most of the MCC genomes, and MCPyV-positive tumor cell growth is dependent on the expression of a viral Large T antigen (LT) with an intact retinoblastoma protein (RB)-binding site. The RB-binding domain in MCPyV LT is – in contrast to other polyomavirus LTs (e.g. SV40) – embedded between two large MCPyV unique regions (MURs). In order to identify elements of the MCPyV LT necessary for tumor cell growth, we analysed the rescue activity of LT variants following knockdown of the endogenous LT in MCC cells. In this regard, we demonstrate that a motif previously described to be a nuclear localization sequence is required neither for MCPyV LT nuclear entry nor for promotion of MCC cell proliferation. Similarly, large parts of the MURs distal to the RB binding domain as well as ALTO – a second protein encoded by an alternative reading frame in the MCPyV LT mRNA – are completely dispensable for MCPyV driven tumor cell proliferation. Importantly, the related SV40 LT – naturally lacking the MURs – as well as MCPyV LT in which the parts of the MURs proximal to the RB-binding domain have been removed can only partially restore MCPyV LT function, implying that a unique growth promoting activity is located in this MUR region. Finally, deletion of the N-terminus of MCPyV LT or a point mutation in this domain, abrogating the interaction with HSC-70, completely abolishes the rescue activity of the protein suggesting that HSC-70 is significantly involved in mediating MCPyV LT function in MCC cells.

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P263 (O17)

Melanoma cell function regulated by VCAM-1 presented on tunable nano-structured surfaces

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Aim: The integrin α4β1 is relevant for melanoma adhesion and migration on VCAM-1, especially when VCAM-1 is up-regulated in endothelial cells during inflammation. However, biophysical parameters of this interaction such as ligand density and spatial distribution are poorly understood. Therefore, by developing innovative artificial matrices with tunable ligand presentation on the nanoscale, we studied VCAM-1-dependent spreading and cellular morphogenesis of human melanoma cells.
Method: Surfaces with precisely tunable densities of the VCAM-1-biomolecule were created using block-copolymer-nanolithography: Glass substrates were covered with nanopatterns of 6 nm gold nanoparticles by self-assembly of diblock copolymer micelles. With this method, the distance between gold nanoparticles could be precisely adjusted between 40 nm and 120 nm. VCAM-1 was then covalently bound in an ortho-directional orientation to the gold nanoparticles resulting in physiologically relevant defined ligand site densities of VCAM-1 molecules covering one order of magnitude (70/m to 670/m).
Results: Integrin α4β1-positive melanoma cells on nanoscopically presented VCAM-1 revealed knew characteristics of this interaction implicated in tumor progression: Nanoscopic presentation of VCAM-1 alone mediated firm attachment but no spreading of melanoma cells, while spreading was readily induced by additional presentation of RGD next to VCAM-1. Second, the morphological properties of melanoma cells on these matrices (VCAM-1+ RGD) were directly regulated by VCAM-1 in a density-dependent fashion with increasing ligand densities significantly inhibiting cell spreading and the according cytoskeletal reorganization. Third, a VCAM-1-density of less than 70/m did not induce any morphological changes – this value possibly representing a numeric cut-off for inducing a cellular response to VCAM-1. The specific dependence of melanoma cell functions on nanoscopically presented VCAM-1 was confirmed by (1) replacing VCAM-1 by a related ligand (PECAM-1), (2) enzymatic degradation of VCAM-1 by elastase and (3) by α4β1-knock-down in melanoma cells. These experimental approaches abrogated the induced effect of nanoscopically presented VCAM-1 proving VCAM-1 specificity.
Conclusions: Our results show that a well characterized receptor/ligand interaction between VCAM-1 and α4β1 is not only controlled by the specificity of the respective binding partners. Rather, biophysical tuning of ligand site densities is an important parameter in the regulation of biological effects – until now not investigated due to the lack of appropriate *in-vitro*-model systems. Such new insights might explain for the first time paradoxical effects described in therapeutical approaches of malignant melanoma going along with variation of VCAM-1 expression/ density in in-vivo situations.

P264

CD4+ T helper (Th1) cell cytokines from freshly generated, melanoma-specific Th1 cells induce senescence in human melanomas

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Recent studies showed that tumor immunotherapy is moving from pre-clinical to clinical studies. Currently the therapy primarily focuses on the treatment of patients with advanced metastatic disease. Two of the therapies, adoptive transfer of killer cells or inhibition of the T-cell silencing signaling molecules (PD-1, CTLA-4) are currently most promising. Yet, both therapies are associated with major toxicity and a significant number of therapeutic failures. An entirely novel approach is immune-mediated cancer paralysis through senescence induction directly in the tumor cells. It was recently shown that the adoptive transfer of tumor-specific CD4+, IFN-gamma-producing Th1 cells can induce senescence in a variety of cancers, but the potential effect and mode of action of IFN-gamma and TNF on melanoma remained enigmatic. To analyze the effects of the combined application of IFN-gamma and TNF, we treated a panel of melanoma cell lines with these two cytokines. FACS-analysis showed that IFN-gamma and TNF can cause apoptosis and various types of cell cycle arrest in the different lines, including a predominant G0/G1 arrest. In a series of melanomas this cell cycle arrest remained stable for >5 passages after withdrawal of IFN-gamma and TNF, a state that defines senescence. Moreover, following stimulation with IFN-gamma and TNF melanoma cells engaged a senescence-associated secretory phenotype with the production of IL-6, IL-8, IP-10 and CCL-2, showing that the two Th1-cytokines induced senescence in a large panel of melanomas, as defined by stable growth arrest and functional phenotype. Based on this we investigated the possibility of generating melanoma-specific Th1 cells capable of driving melanomas into senescence under therapeutic conditions. Therefore we next developed a protocol for the generation of melanoma-antigen specific Th1 lymphocytes. We primed peripheral blood mononuclear cells with a NY-ESO-1 peptide mix and developed stable Th1 cell lines. We then stimulated these expanded CD4+ cells with NY-ESO-1, found that they produced IFN-gamma and TNF and analyzed the effect of this supernatant on the melanoma cell cycle. The supernatant induced both apoptosis and cell cycle arrest, including a G0/G1 arrest as seen in senescent cells. Thus, melanoma-specific Th1 cells can be generated that drive human melanomas in a senescence-defining stable growth arrest. This finding seems to be of great relevance, as modern immunotherapies rather induce stable growth arrest of melanomas and their metastases than complete cancer eradication.

P265

Interferon-gamma-dependent signaling in cancer cell senescence

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p16Ink4a/retinoblastoma protein (Rb)-dependent cell cycle regulation is frequently impaired in various tumors, including HPV-positive epithelial cancers or merkel cell carcinoma. One of the best-established models for these cancers is a RIP-Tag2 mouse expressing the T antigen (Tag) under control of the rat insulin promoter (RIP). In line with human situation, Tag-induced inactivation of p53 and incomplete inhibition of the p16Ink4a/Rb pathway lead to deregulation of the cell cycle and uncontrolled proliferation of the β-cells, and finally to β-cell cancer. In previous studies, we showed that Tag-specific, interferon-γ (IFN-γ)- and tumor necrosis factor (TNF)-producing T helper 1 (TH1) cells strongly reduce β-cell tumor growth thereby doubling the survival of RIP-Tag2 mice. We further demonstrated that this TH1 cell-based antitumor effect is mediated by IFN-γ- and TNF-induced cancer cell senescence in the absence of substantial cancer cell killing. In the present work, we focused our research on IFN-γ-dependent signaling pathways leading to cellular senescence *in vitro*.
First, we determined the gene induction after IFN-γ treatment by PCR array analysis. Here, we repeatedly found up regulation of TNF mRNA after IFN-γ stimulation of the β-cancer cells. These results were confirmed by quantitative rtPCR, and we could show that TNF mRNA is already up regulated after 4 h of incubation. Immune-fluorescence after double staining for TNF and insulin further demonstrated that IFN-γ treatment induced TNF in isolated β-cancer cells. To analyze the functional role of TNF for the IFN-γ-mediated antitumor effect, we treated RIP-Tag2xTNFR1-deficient β-cancer cells with IFN-γ and measured its influence on proliferation and induction of senescence markers. In accordance with a functional role of TNF, RIP-Tag2xTNFR1-deficient cells were less sensitive towards IFN-γ-induced growth inhibition and did not show any signs of IFN-γ-induced senescence, i.e. senescence-associated β-galactosidase activity or nuclear recruitment of pHP1γ. Taken together, our data first demonstrate that IFN-γ-signaling to cancer cells induces TNF in an autocrine loop. They then show that only the combined signaling of the two cytokines arrests βγ-cancer cell proliferation by restoring the cell cycle control. This nontoxic mechanism of cancer control leads to cancer cell senescence, contributes significantly to the cancer immune-surveillance and explains at least in part the protection that interferons provide to patients at increased risk of developing melanoma metastases.

P266

Th1 cell-cytokines arrest cancer through superinduction of the p16Ink4a tumor suppressor

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Many cancers are caused by gene products that deregulate the cell cycle control. Examples are HPV-derived E7, leading to Bowen Carcinoma, Polyoma midle T (PymT), causing Merkel Cell Carcinoma, or Large T antigen (Tag), or BRAFV600E causing melanoma. As T helper 1 (Th1) lymphocytes can efficiently arrest cancer and cause tumor reduction in humans, we analyzed the mode of action, analyzing Tag2-induced carcinomas in mice. First analyses revealed that Th1 lymphocytes control cancer by arresting proliferation of cancer cells *in vivo*, in the absence of enhanced apoptosis or killer-cell-mediated killing. Cancer control strictly required the two cytokines interferon-γ (IFN-γ) and tumor-necrosis-factor (TNF), as in the absence of either cytokine the immune response strongly promoted the cancer growth. While the two Th1 cytokines were unable to kill Tag-expressing cancers, they strongly induced the anti-angiogenic chemokines CXCL9 and CXCL10 and caused an almost complete growth arrest of the cancer cells both *in vitro* and *in vivo*. Detailed cell cycle analyses combined with targeted gene-silencing of cell cycle genes with short hairpin RNA (shRNA), revealed that the combined action of IFN-γ and TNF restored the cell cycle control mechanisms disturbed by Tag, through super-induction of the tumor suppressor protein p16Ink4a in cancer cells. To separate the senescence-inducing effects from anti-angiogenesis we isolated cancer cells from the pancreas and down-regulated p16INK4a by shRNA. While low levels of p16INK4a did not affect the chemokine production, it completely abrogated the capacity of IFN-γ and TNF to arrest the cell cycle. As senescent cancer cells remained growth arrested even when transplanted in severely immune compromised mice, while sham-treated cancers grew rapidly. As IFN-γ and TNF also induced cell cycle arrest and senescence in a large panel of cancers of human or mouse origin and as human cancers regress in the cytokine storm induced by immune stimulation with antibodies directed against CTLA4 and PD1/PD1L cytokine-induced senescence seems to be a basic mechanism underlying cancer control by the immune system.

P267 (O23)

Metastasis suppressor 1 (MTSS1): a metastasis driver in a subset of human melanomas

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Background: Profound complexity of cancer genome and associated multiple disease mechanisms have undermined the success of anti-cancer approaches, including novel 'targeted' ones. Even so activating BRAF(V600E) remains the earliest, most frequent, and therapeutically most pursued oncogenic driver of melanomagenesis, identification, characterization and targeting of less frequent (subgroup-associated), yet functionally critical metastatic driver alterations could be key to sustained therapeutic responses.
Observations: We used a sequential search across human tumor samples for transcript outlier data points with associated gene copy number variations that are correlated with patient's survival to identify genes with proinvasive functionality. Utilizing loss (siRNA-mediated RNA interference)- and gain-of-function (gene overexpression) approaches in appropriate biological assays, we reveal MTSS1 as a positive regulator of melanoma cell migration and invasion. Notably, MTSS1 overexpression in the cells of melanocytic origin significantly enhanced their *in vivo* tumorigenic and metastatic potential. While MTSS1-WH-2 domain was dispensable for the invasive properties engendered by MTSS1, Rac-interacting MTSS1-I-BAR domain was critical to this functionality. Through siRNA-mediated silencing, we show active cofilin as a critical effector of MTSS1-mediated melanocytic cell migratory enhancement. We further demonstrate that MTSS1 engages and suppresses RhoA-ROCK-LIMK and Rac-PAK-LIMK signaling to induce cofilin activity. Lastly, we reveal that high MTSS1 expression levels were indeed associated with a subgroup of primary melanomas showing unfavorable prognosis.
Conclusions: While offering a systematic framework for unravelling the low frequency metastatic driver events in malignancies of varied origins, the identification and characterization of MTSS1 as a melanoma subgroup-associated driver of metastatic program could offer potential therapeutic opportunities.

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VEGF-A serves cell-type specific functions in HPV8-mediated carcinogenesis and coordinates angiogenesis-dependent and -independent mechanisms of hyperplastic growth

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Human papillomaviruses (HPV) of genus beta are suspected carcinogenic in nonmelanoma skin cancer (NMSC). However, the mechanism of action remains a challenge. The strongest evidence for a causal role in the development in squamous cell carcinoma (SSC) exists for HPV8 in patients with epidermodysplasia verruciformis.
To gain insight into the molecular mechanisms underlying HPV8-mediated skin tumor development, we previously developed HPV8 transgenic mouse models that recapitulate the HPV8-induced SCC pathology and have been proven to be a valuable *in vivo* model to unravel the molecular pathology of HPV-induced skin cancer. It is not entirely understood, how HPV8 infected keratinocytes escape cell cycle control and whether their crosstalk with immune cells may contribute to tumor formation. The angiogenic cytokine Vascular endothelial growth factor-A (VEGF-A) has been identified as critical regulator of tumor development both through induction of tumor angiogenesis but also via angiogenesis-independent mechanisms. Up to date, the role of VEGF-A in HPV-induced NMSC is not resolved, neither the question whether diverse cellular sources of VEGF-A may impact this process.
In this study we dissected the contribution of epidermis- versus myeloid cell-derived VEGF-A in HPV8-mediated skin cancer using a combination of HPV8 transgenic mice and conditional gene targeting for VEGF-A. Here we show, that epidermis-specific deletion of VEGF-A results in complete abrogation of tumor initiation in HPV8 mice both spontaneous and in the presence of diverse tumor

promoting conditions. In contrast, myeloid cell-derived VEGF-A is only critical for tumor formation triggered by full thickness excision skin injury. Mechanistically, we show that blocking VEGFR2 inhibited injury-induced papilloma formation in HPV8 transgenic mice, indicating an important paracrine function of VEGF-A in tumor angiogenesis. Furthermore, our findings provide evidence that epidermal HPV8 proteins can deviate a primarily beneficial and healing-promoting acute inflammatory response into a sustained inflammatory response leading to hyperplastic growth, and that myeloid cell-derived VEGF-A plays a critical role in this process. Unexpectedly, reduced clonal growth of VEGF-A depleted keratinocytes *in vitro* could not be rescued by external rVEGF-A, suggesting an additional cell-autonomous activity of VEGF-A in keratinocytes, independent from angiogenesis.
Collectively, here we provide novel mechanistic insights in distinct functions of epidermal- versus myeloid cell-derived VEGF-A in HPV8-mediated tumor development, which may have important implications for the prevention and treatment of HPV-mediated skin cancer.

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XIAP down-regulation reduced migration and invasion of human metastatic melanoma cells

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XIAP belongs to the IAP inhibitors of apoptosis protein family (c-IAP1, c-IAP2, and XIAP) whose expression is significantly increased in several cancers including melanoma. The activity of these molecules is intracellularly regulated by endogenous inhibitors among which a relevant role has been shown for the second mitochondria derived activator of caspase Smac protein whose expression has also been detected in human melanomas. *In vitro*, XIAP and Smac were expressed in melanoma cell lines of high invasive grade, MeWo, A375 and BLM cells, with higher expression in A375 and BLM. All cells were able to efficiently invade dermal skin equivalents *in vitro* and inhibition of IAP molecules using Smac mimetics led to a more significant inhibition of BLM invasion as compared to MeWo and A375.
To further address the role of XIAP expression for BLM invasive abilities, we have used a RNA interference approach and have stably silenced XIAP expression in these cells (sh-XIAP BLM). XIAP down-regulation did not affect expression of the other cIAPs, indicating that no compensation by these occurred. Moreover, additional *in vitro* analysis revealed significantly reduced cell proliferation rate in sh-XIAP BLM as compared to control clones (scrambled vector transfected), which however did not result from increased apoptosis rate. Interestingly, migration of sh-XIAP BLM cells on fibronectin coated surfaces was reduced likely as consequence of reduced cellular organization on this substrate. Indeed, sh-XIAP BLM cells when plated on fibronectin failed to organize their actin filament network and displayed reduced recruitment of vinculin at cellular borders as compared to control clones displaying stress fibers and extensive vinculin localization in focal adhesion points at the cellular membrane. Importantly, as a result from all these molecular alterations, XIAP down-regulation in BLM cells led to a significant decrease in invasion of dermal skin equivalents.
Taken together, XIAP down-regulation in BLM cells reduced proliferation, migration and subsequently invasion of metastatic melanoma cells *in vitro*. Further studies will aim to investigate the molecular links existing between XIAP and the migratory capacity. These results indicate that XIAP could be a pro-metastatic gene in skin melanoma and qualify as a therapeutic target for anti-cancer treatments.

P270

Y-box binding protein 1 – key player in melanoma cell proliferation and progression

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Y-box binding protein 1 (YB-1) is a multifunctional protein involved in various cellular processes including both transcriptional and translational regulation of target gene expression. Significantly increased YB-1 levels have been reported in a number of human malignancies and shown to be associated with poor prognosis and disease recurrence.
Our previous data indicated that YB-1 is upregulated during melanoma progression and that downregulation of YB-1 levels impedes proliferation, survival, migration, invasion and chemosensitivity of metastatic melanoma cells. With this study we now focused on the functional effects of YB-1 overexpression in tumour cell proliferation and progression at different stages of melanoma development. Interestingly, total YB-1 levels could be altered only to a rather small extent indicating a tight regulation of tumour stage specific YB-1 expression in melanoma cells by means of feedback mechanisms. Based on previous findings postulating a mitogenic function of extracellular YB-1 in both an inflammatory and a breast cancer setting, we further analysed the occurrence and the relevance of secreted YB-1 in terms of melanoma progression as well as its potential role as a tumour marker. Moreover, the role of increased YB-1 levels in mediating vemurafenib resistance was evaluated.

P271

Loss of ERBB3 in mouse skin decreases tumor growth during multi-stage chemical carcinogenesis

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Gain- and loss-of-function studies in genetically modified mice established the epidermal growth factor receptor (EGFR, ERBB1, HER1) and its ligands as important regulators of keratinocyte proliferation and differentiation, with implications for wound healing, skin inflammation and carcinogenesis. Less is known about the functions of the structurally related tyrosine kinase receptor ERBB3 (HER3), a member of the ERBB receptor family, in this tissue. ERBB3 is co-expressed with the EGFR in the epidermal granulosa layer and in several cell types of the pilosebaceous unit. Interestingly, ERBB3 is overexpressed in numerous types of cancers, including some human non-melanoma skin cancers.
To investigate the functions of ERBB3 in the skin, we crossed mice carrying a conditional Erbb3 allele with transgenic animals expressing cre recombinase under the control of the keratin 5 (K5) promoter. Recombination of the Erbb3 allele and loss of ERBB3 in the skin, with unchanged receptor levels in other organs, was confirmed by PCR and immunohistochemistry, respectively. K5Cre;Erbb3del mice were born at the expected ratios and showed no obvious abnormalities, strongly indicating that ERBB3 is dispensable for the development and the homeostasis of the epidermis and its appendages. Next, to analyze the function of ERBB3 during tumorigenesis, we employed a multi-stage chemical carcinogenesis protocol. Seven-week-old K5Cre;Erbb3del females and control littermates received a single application of the initiating agent 7,12-dimethylbenz(a)anthracene followed by multiple applications of the promoting agent 12-O-tetradecanoylphorbol-13-acetate for several weeks. We found that ERBB3 knockout mice remained free of papillomas for a longer time than control littermates. Also, the tumor burden was significantly increased in ERBB3-deficient mice. The present data indicate that ERBB3 signaling contributes to tumor growth during multi-stage chemical carcinogenesis in mice.
To investigate the mechanisms behind this observation in more detail, we are complementing our studies with cell culture experiments using the human epidermal cell line HaCaT and the human epidermal carcinoma cell line A431. Specifically, we aim to investigate the consequences of ERBB3 loss or overexpression on parameters such as proliferation, migration, and signaling.

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ROS-dependent phosphorylation of Bax by wortmannin sensitizes melanoma cells for TRAIL-induced apoptosis

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The pathways of reactive oxygen species (ROS)-mediated apoptosis induction, of Bax activation and the sensitization of tumor cells for TRAIL-induced apoptosis remained largely elusive. Here, sensitization of melanoma cells for TRAIL by the PI3-kinase inhibitor wortmannin correlated to activation of mitochondrial apoptosis pathways. Apoptosis was dependent on Bax and abrogated by Bcl-2 overexpression. The synergistic enhancement was explained by Bax activation through wortmannin, which tightly correlated to characteristic Bax phosphorylation patterns. Thus, wortmannin resulted in early reduction of the Bax-inactivating phosphorylation at serine-184, whereas the Bax-activating phosphorylation at threonine-167 was enhanced. Proving the responsibility of the pathway, comparable effects were obtained with an Akt inhibitor (MK-2206). While suppressed phosphorylation of serine-184 may be attributed to reduced Akt activity itself, the causes of enhanced threonine-167 phosphorylation were addressed here. Characteristically, production of ROS was seen early in response to wortmannin and MK-2206. Providing the link between ROS and Bax, we show that abrogated ROS production by alpha-tocopherol or by NADPH oxidase 4 (NOX4) siRNA suppressed apoptosis and Bax activation. This correlated with reduced Bax phosphorylation at threonine-167. The data unravel a mechanism by which NOX4-dependent ROS production controls apoptosis via Bax phosphorylation. The pathway may be considered for proapoptotic, anticancer strategies.

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The BH3-only protein BimL overrides Bcl-2-mediated apoptosis resistance in melanoma cells

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Melanoma cells are characterized by apoptosis deficiency coinciding with reduced expression of the proapoptotic Bcl-2 protein Bim. An adenoviral vector was constructed with the BimL cDNA controlled by an inducible promoter. Highly efficient apoptosis induction and abrogated cell proliferation was seen in melanoma cells upon BimL overexpression. Loss of mitochondrial membrane potential, release of mitochondrial apoptogenic factors and caspase-9 processing indicated the activation of mitochondrial apoptosis pathways. BimL activated both Bax and Bak, as shown by siRNA knockdown and activation-specific antibodies. Of note, BimL overrode the apoptosis blockade by Bcl-2 overexpression or by Bax/Bak single knockdown. The high efficacy correlated to BimL interaction with all antiapoptotic Bcl-2 family members in melanoma cells, shown by co-immunoprecipitation analyses for Bcl-2, Bcl-xL, Mcl-1 and Bcl-w. Thus, BimL reveals an outstanding proapoptotic potential in melanoma cells, and strategies for its re-expression appear of interest. These have been reported for B-Raf inhibitors, and their efficacy may be partly attributed to BimL.

P274

STAT1-signaling in cancer cells critically shapes carcinogenesis

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Immunotherapy with tumor-specific T-helper-1 (Th1) cells has been shown to reduce tumor burden in humans with melanoma and in mice with neuroendocrine cancers. Previous data showed that this tumor control resulted from the interferon-γ (IFN-γ)- and tumor necrosis factor (TNF)-mediated super-induction of the p16Ink4a tumor suppressor. Induction of p16Ink4a causes a stable growth arrest called senescence and is completely abrogated *in vivo* by anti-IFN-γ mAb. To determine the mechanisms underlying the IFN-γ- and TNF-mediated growth arrest in cancers, we studied islet carcinomas in RIP-Tag2 mice. Here, expression of SV40 large T antigen 2 (Tag2) under the rat insulin promoter 1 (RIP) disturbs the cell cycle control through inhibition of p53 and Rb1. To analyze the IFN-γ-signaling, we generated RIP-Tag2 mice deficient in STAT1, a main IFN-signaling pathway. While Th1 cells were capable of doubling the life span of RIP-Tag2 mice, Th1 cells failed to prolong the survival of RIP-Tag2xSTAT1.ko mice. In line with this, islet cancers grew and blood glucose decreased as rapidly as in sham-treated RIP-Tag2 mice, whether mice were sham-treated or treated with Th1 cells. Surprisingly, histology of the cancers revealed that cancers grew more aggressively in RIP-Tag2xSTAT1.ko mice and roughly 50% of RIP-Tag2xSTAT1.ko mice developed macroscopic metastasis within 12 weeks, while STAT1-competent RIP-Tag2 mice never developed metastases (<1%). As these data suggest that STAT1-expression in cancers controlled the epithelial to mesenchymal transition (EMT), we compared gene expression pattern of tumors *ex vivo*. These data underlined that STAT1 suppresses cell motility and invasiveness through induction of epithelial differentiation marker like E-cadherin, Keratin-7 or Desmoplakin, and suppression of the mesenchymal marker Foxc2. To determine whether this enhanced EMT and risk of spreading was an intrinsic defect of STAT1-deficient cancer cells or whether it was caused by the cancer environment, we generated pure β-cancer cell lines from either RIP-Tag2 or RIP-Tag2xSTAT1.ko mice and injected the cancers into NOD-SCID.IL2Rcγ.ko mice. Again, RIP-Tag2xSTAT1.ko cancers grew more rapidly and decreased the serum insulin levels more rapidly than STAT1-competent RIP-Tag2 cancers. *In vitro* analyses confirmed that the combined action of IFN-γ and TNF efficiently super-induced the p16Ink4a tumor suppressor and growth-arrested normal RIP-Tag2 cancers, while they failed to do so in RIP-Tag2xSTAT1.ko cancers. Thus, STAT1-signaling in cancer cells was critically needed for the IFN-γ- and TNF-mediated growth arrest in cancer cells. As, in addition, loss of STAT1 in cancers promoted EMT and resulted in more aggressive cancer growth, the data uncover critical signaling pathways required for interferon-mediated cancer control.

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Hidden biomechanics: new insights into the role of growth factors VEGF-A/VEGF-C in a subcutaneous murine xenograft model of angiogenesis

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Solid tumors when grown in a xenograft mouse model are usually known to exhibit an elevated tumor interstitial fluid pressure (TIFP), thus hampering efficient uptake of chemotherapeutical drugs of high molecular weight at their target location. This phenomenon can be attributed in part to the chaotic organization of the tumor vessel network as well as an internally impaired lymph fluid drainage commonly found in tumor types such as in the A431 vulva-carcinoma derived xenografts. Here, we attempt to investigate systematically the influence that different concentrations of VEGF-A and VEGF-C exert on (lymph) vessel formation, apoptosis, proliferation levels and the development of TIFP. We found that a peak optimal concentration of 1 μg/ml VEGF-C, applied peritumorally, can significantly increase lymph vessel formation and correlates with a reduction of TIFP. Additionally, we use a new

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approach of combined plastination/maceration and μCT techniques to display vessel architecture as a 3D-model with micrometer resolution. With this technique, we aim to compare critical parameters such as tumor vessel branching, size, length and radial distribution patterns for different tumor types and treatments.

P276 (O35)

Ultraviolet radiation-induced neutrophilic inflammation promotes angiotropism and metastasis in melanoma

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Background: Intermittent intense ultraviolet (UV) exposure represents an important etiologic factor in the development of malignant melanoma. With the advent of next generation sequencing technologies the ability of UV radiation to cause tumour-initiating DNA mutations in melanocytes is now firmly established. It has been hypothesised that the effects of UV radiation on epidermal keratinocytes and immune cells additionally promote melanoma development by stimulating the survival, proliferation and migration of DNA-damaged melanocytes. How these microenvironmental effects of UV irradiation influence melanoma pathogenesis is incompletely understood.
Methods: We experimentally investigated the impact of UV-induced inflammatory responses on the progression of incipient primary melanomas that were initiated by one epicutaneous application of the carcinogen DMBA in HGF-CDK4(R24C) mice as well as on serial HGF-CDK4(R24C) melanoma skin transplants in syngeneic wild type mice. These model systems allowed us to study the tumor-promoting effects of UV irradiation independent of its tumor-initiating effects.
Results: Repetitive UV irradiation induces a neutrophil-rich skin inflammatory response that did not alter the incidence, multiplicity and growth kinetics of DMBA-induced primary cutaneous melanomas in a cohort of 20 HGF-CDK4(R24C) mice compared to 20 non-irradiated controls. However, detailed pathological analyses of mice with progressively growing melanomas revealed that UV irradiation enhanced the expansion of tumor cells along blood vessel endothelial cell surfaces in a pericyte-like manner which occasionally became macroscopically visible in the dermis. This phenomenon was originally described as angiotropic growth by histopathologists in human melanomas. Consistent with observations in the human system, we found that enhanced angiotropism was associated with significantly increased numbers of lung metastases. UV irradiation of mice bearing serial HGF-CDK4 (R24C) melanoma skin transplants also enhanced angiotropism and increased the number of spontaneous metastases in the lung. These results in a very controlled experimental setting recapitulated our findings with DMBA-induced primary melanomas. Importantly, we found that antibody-mediated depletion of Ly6G+ neutrophils abrogated the metastasis-promoting effects of UV irradiation.
Conclusions: Taken together, our work provides evidence that repetitive UV-irradiation induces a neutrophilic inflammatory response which catalyses reciprocal melanoma-endothelial cell interactions and drives angiotropic growth, e.g. the perivascular invasion of melanoma cells. Angiotropism represents a hitherto underappreciated mechanism of metastasis, which also increases the likelihood of intravasation and dissemination via the blood. A better understanding of inflammation-induced interactions between tumor and endothelial cells may lead to new treatment approaches that impair metastatic progression of high-risk primary melanomas.

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Functional role of Ngfr signaling in human melanoma cell lines

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Phenotypic heterogeneity in malignant tumors has often been attributed to hardwired genetic changes in distinct tumor cell clones. However, recently it has been demonstrated for a number of cancers – including malignant melanoma- that reversible adaptive plasticity of tumor cells contributes considerably to intratumoral heterogeneity and poses an important mechanism for therapy resistance.
In our previous work we could show that melanoma cells exist in a dynamic, interconvertible equilibrium between differentiated and dedifferentiated subpopulations that rapidly adapts to inflammatory signals in the environment. We identified TNF-alpha as an important modulator of this phenomenon and demonstrated that it potently upregulates the neural crest marker Ngfr (CD271) on melanoma cells.
We therefore hypothesizd that the upregulation of Ngfr on human melanoma cells may play an important role for survival and progression of tumor cells under proinflammatory conditions.
We investigated the expression of Ngfr and its classical ligands – the four neurotrophins Ngf, Bdnf, NT-3, NT-4/5 – on a larger panel of human melanoma cell lines by PCR, FACS analysis and ELISA under normal and proinflammatory culture conditions. We also assessed the effect of Ngf on proliferation, survival and migration of human melanoma cells.
We could confirm our findings that TNF-alpha treatment leads to a reversible phenotype shift to less differentiated Ngfrhigh melanoma cell subpopulations in a large panel of human melanoma cell lines. In contrast to data in the literature we could not observe autocrine secretion of neurotrophins by melanoma cells as ligands for Ngfr. We could demonstrate that the neurotrophin Ngf leads to enhanced migration of melanoma cells and pre-treatment with TNF-alpha further enhances these migratory capacities.
Taken together we could show that the neurotrophin Ngf is a chemo-attractant for human melanoma cells. TNF-a treatment endows melanoma cells with migratory capacities and further enhances migration in response to Ngf. We currently investigate possible physiologic sources for Ngf under normal and proinflammatory conditions. We are also dissecting the pathways that may underlie melanoma cell migration towards Ngf with special focus on its receptors Ngfr and TrkA.

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Defining the mode of melanoma's proliferative heterogeneity by real-time cell cycle imaging

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Solid cancers, including melanoma, are typically composed of irregular zones containing both actively cycling and quiescent cells. Despite considerable insight into the molecular mechanisms underlying aberrant cancer cell cycle progression, there is limited understanding as to what regulates the positioning of proliferating or quiescent cancer cells within the complex tumor microenvironment. Moreover, the relationship between cancer cell invasion and cell cycle progression is poorly understood. Here, we utilized the fluorescent ubiquitination-based cell cycle indicator (FUCCI) to longitudinally monitor proliferation and migration of melanoma cells in three-dimensional cell culture and in situ. We found that melanoma cells in a hypoxic microenvironment or in the presence of MAPK inhibitors remained reversibly arrested in G1 for long periods of time. We further demonstrated that invading melanoma cells cycled actively, even after inhibition of the microphthalmia-associated transcription factor (MITF), which caused increased invasiveness. Melanoma xenografts displayed two distinct proliferative architectures characterized by differential distribution of cycling cells, which was regulated by MITF. While MITF-high melanomas revealed a phenotype characterized by a more random distribution of proliferating cells, MITF-low melanomas were composed of proliferation hot spots and areas of G1-arrested cells. Downregulation of MITF using shRNA in MITF-high melanomas reversed the phenotype. Taken together, our data challenge the idea that the invasive and proliferative capacity of melanoma cells are mutually exclusive, and uncover the importance of MITF in the proliferative behavior of melanoma cells within the tumor microenvironment.

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Induction of endoplasmic reticulum stress as a strategy for melanoma therapy

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We have shown that melanomas are composed of differentially cycling tumour cells in a subcompartment-specific distribution, which may result in differential sensitivity to apoptosis. Further, we have demonstrated that targeting the endoplasmic reticulum (ER) with fenretinide (synthetic retinoid) or bortezomib (26S proteasome inhibitor) induces cell cycle arrest and apoptosis of metastatic melanoma cells *in vitro* and *in vivo*. This study aims (1) to investigate the effect of ER stress-inducing drugs on the dynamics of cell division and cell death of individual melanoma cells within the tumour microenvironment, and (2) to develop combination strategies that increase the efficacy of ER stress-inducing agents for the treatment of melanoma.
We made use of the fluorescent ubiquitination-based cell cycle indicator (FUCCI), which facilitates real-time cell cycle tracking. We utilised the F-XBP1ΔDBD-venus reporter construct, which labels the cytoplasm in response to ER stress.
We show that bortezomib-induced ER stress, delayed cell cycle progression, and combination with fenretinide increased cell death in our 3D melanoma model. While the selective BRAF-inhibitor vemurafenib induced G1 arrest, bortezomib induced G1- and G2 arrest, but preferentially killed G2-phase cells. Temozolomide enhanced the effect of bortezomib. However, MEK inhibitors blocked the effect of bortezomib in all melanoma cells, as did BRAF inhibitors in BRAF mutant cells.
Our data suggest that bortezomib combined with fenretinide or temozolamide is a therapeutic strategy worth exploring for the treatment of BRAF-inhibitor insensitive or resistant melanoma. Importantly, melanoma cells in G1 are protected from the cytotoxic effect of bortezomib, which excludes MAPK inhibitors as combination partners.

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Rose Bengal – phototoxicity versus intrinsic cytotoxicity

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Rose Bengal (4,5,6,7-tetrachloro-2′,4′,5′,7′-tetraiodofluorescein disodium salt; RB) is a fluorescent compound that has been in use in ophthalmology in the diagnostics of corneal damage. RB is a photosensitiser and its phototoxicity is well characterised. Recently, it has been tested as an intralesional agent (in the absence of light) for the treatment of cutaneous melanoma metastases and is currently undergoing further testing in a Phase II trial. However, the mechanism of action of RB on melanoma in the absence of light is not thoroughly understood.
In addition to standard assays such as 2D drug sensitivity assays, DNA content analysis, Annexin V staining, immunoblotting and confocal microscopy we also made use of a number of more unique assays. We transduced melanoma cells with fluorescently labelled LC3 to visualise the accumulation of LC3-II in the membrane of autophagosomes. Further, we combined the fluorescent properties of RB with live/dead stains to perform three-colour fluorescence imaging of our 3D melanoma spheroids.
RB indeed had a dosedependent cytotoxic effect on melanoma cells but not fibroblasts in the absence of light or upon exposure to red light (633 nm). In contrast, exposure to UV- or green light (561 nm) caused profound phototoxicity within minutes. In our 3D melanoma spheroid model, RB had a time- and dose-dependent effect on melanoma cell death of both proliferating and invading cells. In addition, RB exerted its toxicity through necrosis without perturbation of the cell cycle and the effects observed in the dark were independent of the phototoxic generation of ROS. Finally, we showed that RB induced autophagy in melanoma cells indicating a possible mechanism of action.
In addition to its phototoxicity RB also exerts intrinsic cytotoxicity. In contrast, to the phototoxicity the intrinsic cytotoxicity has a wider therapeutic window. Here we showed that an interplay of cell necrosis and autophagy is one possible mechanism of action for RB.

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Tumour interstitial fluid pressure (TIFP): a biophysical phenomenon that affects the expression of ADAMTS1 in a xenograft tumour model

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Great efforts have been made to increase the specificity of targeted therapeutics in the fight against cancer; there are, however barriers that limit uptake in the desired tumour site. The central protagonist that counteracts the enrichment of pharmacological compounds within solid tumours is their enhanced TIFP. It has been shown that a high TIFP is a general property of solid tumours in experimental animals and humans. The origin of TIFP is mainly attributed to lymph-vessel abnormalities, abnormal vasculature and the highly permeable blood-vessel network in the tumour area.
The present experimental set-up focuses on the identification of the expression patterns of ADAMTS1 and HIF-1α mRNA and proteins in tumours with different TIFP values. The experiments are performed *in vitro* and *in vivo* utilizing three distinct tumour cell lines (A431, A549 and MDA-MB-231). While the *in vitro* experiments focus on the effect of hypoxia on ADAMTS1, the *in vivo* investigations are performed to obtain data how different TIFP values contribute to the expression of ADAMTS1 and HIF-1α.
In conclusion, we identified distinct expression patterns for ADAMTS1 mRNA and protein in the *in vitro* experiments comparing 3 different cancer cell lines. *In vivo* data highlighted that the mRNA expression showed significantly different expression profiles compared to the ADAMTS1 protein expression pattern in xenograft tumours. These are the first data obtained to investigate the expression of ADAMTS1 in different tumours which differ in TIFP values.

P282

The biological functions of IGF1R signaling in melanoma

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Insulin-like growth factor receptor 1 (IGF1R) signaling is considered to be important in tumor initiation and progression. In this project we analysed the mechanism involved in regulation of IGF1R expression and IGF1R mediated therapy resistance of melanoma cells towards vemurafenib. Our results show that in human melanoma cells the expression of IGF1R positively correlates with PTEN expression. Lentivirus mediated re-expression of PTEN in PTEN loss melanoma cells can induce enhanced IGF1R expression, but inhibition of PI3K/AKT pathway just slightly increases IGF1R expression. Moreover, sensitivity of human melanoma cells to IGF1R inhibition positively correlates with PTEN expression. Interestingly, we found that vemurafenib induces IGF1 expression in human BRAFV600E melanoma cells and down regulates the expression of IGF1R. Inhibitory siRNA mediated knock down of IGF1R can sensitize BRAFV600E melanoma cells to vemurafenib treatment. Vice versa, over expression of IGF1R confers BRAFV600E melanoma cells resistance to vemurafenib treatment. In addition, dermal primary fibroblasts are able to increase BRAFV600E melanoma cell resistance to vemurafenib by a cell-cell contact dependent stabilization of IGF1R expression on melanoma cells. Taken together, IGF1R expression in melanoma cells is regulated by PTEN in a PI3K/AKT signaling pathway dependent and independent way. Furthermore, melanoma and fibroblast cell-cell contact mediates increased resistance towards the BRAFV600E inhibitor vemurafenib. Our data indicate that IGF1R expression in melanoma cells is regulated not only by paracrine mechanisms, but also by heterologous cell-cell contact and by tumor cell intrinsic mechanisms.

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Characterisation of slowly cycling melanoma cells as major originators of melanoma plasticity, tumorigenicity and therapy resistance

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Enormous efforts have been made within the last decade in order to improve therapy and survival of stage IV melanoma patients. However, the current therapeutics failed to perform curative outcomes, indicating to a cancer stem cell model where only the tumour bulk is responding to the treatment. Melanoma tumours seem to be organized in a more complex manner as proposed by the classical hierarchical or clonal evolution cancer stem cell models resulting in 'dynamic stemness'. This seems to be mainly due to the high plasticity of malignant melanoma implying that every melanoma cell can turn into a tumour initiating or melanoma stem cell. Therefore, it seems to be essential 1st to proof the concept of dynamic stemness for melanoma and 2nd to characterize these cells in respect of signaling, tumourigenicity and therapy resistance.

First, we assayed the prevalence of slowly cycling melanoma cells under different culture conditions namely monolayer culture, 3D spheroids and melanoma sphere cultures using CFSE label retaining. Label retaining respectively slowly cycling cells were found in increasing abundance from monolayer culture to three dimensional cultivation techniques like spheroids and spheres.

In a second step we isolated these cells by fluorescence activated cell sorting (FACS) and tested their potential for clonogenic growth and responsiveness to standard melanoma therapeutics. Slowly cycling cells were further tested by western blot for their MAPK, PI3K and beta-catenin signalling activity in comparison to normal cycling cells.

Third, we evaluated interconversion of CFSE retaining cells and CFSE diluting cells in order to proof the hypothesis of the 'dynamic stemness model' for malignant melanoma.

Further characterisation and identification of the factors that are essential for the formation of this stem-like melanoma cell population might be beneficial for future therapy approaches yielding better and persistent therapy responses.

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Molecular mechanisms in stem cell-driven skin tumour initiation

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Mammalian skin functions as a barrier between the body and the external environment and is constantly assaulted by genotoxic stress such as UV irradiation. Recent studies have shown that epidermal stem cells, which are crucial for maintaining skin homeostasis, respond differently to stress and DNA damage compared to their rapidly cycling progeny. In particular, multipotent hair follicle stem cells are more resistant to DNA-damage-induced cell death than other cells of the epidermis. This has been linked to a higher expression of the pro-survival factor Bcl2 and attenuated p53 activation as a consequence of faster DNA repair activity in stem cells (Sotiropoulou et al 2010).

Here, we identify an important role of these crucial stem cell surveillance mechanisms in the process of tumour initiation. More specifically, we generated an inducible skin tumour mouse model and have shown that expression of the mutant transcription factor Lef1 induces stem cell-driven sebaceous tumours. Detailed analysis of molecular mechanisms potentially contributing to tumour formation demonstrated that expression of mutant Lef1 disturbed the normal p53 response in hair follicle stem cells. In addition, mutant Lef1 induced an accumulation of DNA damage in stem cells. As a consequence and due to a loss of the pro-survival factor Bcl-2, an increase of apoptosis of hair follicle bulge stem cells was detected. To compensate stem cell loss, proliferation was stimulated within the bulge stem cell compartment.

Thus, our data suggest a tight regulation of apoptosis and proliferation in hair follicle bulge stem cells to sustain tissue homeostasis. However, mutant Lef1 allows for proliferation of stem cells possessing low levels of DNA damage and therefore results in propagation of cells that escaped normal control of proliferation and DNA repair, finally leading to tumour initiation. The results demonstrate that mutant Lef1 interferes with stem cell-specific surveillance mechanism and show for the first time that a tight control of these crucial mechanisms is required to prevent tumourigenesis.

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Human mitochondrial DNA deletions are significantly decreased *in vivo* in melanoma with tumor thickness larger than 3 mm compared to nevi and melanomas with tumor thickness smaller than 1 mm isolated by laser capture microdissection

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High frequencies of the mitochondrial 4977 bp so called common deletion are associated with aging especially photoaging, but a possible role of this deletion in tumors is still discussed controversial, with reports of varying deletion frequencies in different tumors compared to control tissue. The frequencies of the common deletion in melanomas as wells as nevi are still unknown. Especially dysplastic nevi are an important risk factor for the development of melanoma. The aim of this study was to investigate the frequency of the common deletion in melanomas of initial and advanced stages and in normal and dysplastic nevi. To accomplish this, samples were collected from four different groups of patients with normal nevi, dysplastic nevi, initial melanomas with tumor thickness smaller than 1 mm and advanced melanomas with tumor thickness larger than 3 mm. Each group consists of at least 20 patients and tissue adjacent to the lesion of each individual patient of each group was used as control. Tumor and non-tumor samples were confirmed by an independent pathologist, hematoxylin eosin stained and then laser capture microdissected with a Laser Capture Microdissection (LCM2105) device to avoid contamination with adjacent tissues of each sample. Subsequently, DNA extraction and real time PCR for detection of the 4977 bp common deletion was performed. The frequency of common deletion in each sample was normalized to the respective control tissue of each individual patient. In most of the normal nevi, the dysplastic nevi and initial melanomas the frequency of the common deletion was higher when compared to the respective adjacent control tissue. The average frequency of common deletion in nevi samples was approximately 51 times higher than in adjacent control tissue. This tendency was also visible in the group with dysplastic nevi (with an average increased frequency of approximately 93 times) and in the group with initial melanomas (with an average increased frequency of approximately 100 times). Interestingly the frequency of common deletion decreased dramatically to an average decreased frequency of approximately 0.5 times compared to adjacent control tissue. This decrease in the frequency of the common deletion was highly significant, when compared to the frequencies of nevi and initial melanoma. This finding indicates a selection mechanism in melanoma against high levels of common deletion and its depletion in advanced melanomas could serve as a tumor marker.

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Hypoxia confers resistance towards ascorbate-induced ROS-driven cytotoxicity in melanoma cell lines

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Intravenous applications of high-dose ascorbate are used in complementary medicine to treat terminal cancer patients. Pharmacological doses of ascorbate in the millimolar range induce cytotoxicity in cancer cells mediated by reactive oxygen species (ROS), namely hydrogen peroxide and ascorbyl radicals. However, little is known about intrinsic or extrinsic factors modulating this ascorbate-mediated cytotoxicity.

Under normoxia and hypoxia ascorbate, IC50 values were determined on the NCI60 cancer cells, among those seven melanoma cell lines. The cell cycle and GLUT-1 (a pro-survival HIF-1α-downstream target) expression were analyzed after ascorbate exposure under normoxic and hypoxic conditions.

The amount of ascorbyl radicals generated increased with rising serum concentrations. Hypoxia (0.1% O2) significantly increased the cytotoxic IC50 of ascorbate in the melanoma cell lines, thus inducing cellular resistance towards high-dose ascorbate. This did not correlate with cell line-specific expression of the ascorbate-transporter GLUT-1 but was highly dependent of hypoxia and HIF-1α-signalling as determined by CoCl2 treatment. However, under hypoxic conditions, ascorbate treatment at the individual IC50 reduced the expression of GLUT-1 in the cancer cells.

Our data show a ROS-induced, serum-catalyzed and O2-dependent cytotoxicity of ascorbate on 60 different cancer cells which can be attenuated by HIF-1α. This suggests that for clinical application, cancer patients should be oxygenized in order to raise the efficacy of high-dose ascorbate beyond a cytotoxic threshold.

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RAGE ligands S100A8/A9 and HMGB1 as prognostic markers in melanoma

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Early detection of melanoma progression by biomarkers is key to guide treatment decisions and to improve outcome in asymptomatic patients with metastases. The standard melanoma biomarker S100B lacks sensitivity in detecting melanoma progression in up to 20% of patients.

We have recently demonstrated that the receptor for advanced glycation end-products (RAGE) is a central driver of inflammation-associated tumorigenesis by sustaining a chronic inflammatory tumor-microenvironment.

This study was aimed to identify new prognostic biomarkers of melanoma by a hypothesis-driven approach linking the RAGE pathway with clinical outcome of melanoma patients. Moreover, the new candidates, the soluble form of RAGE (sRAGE) and the RAGE ligands S100A8/A9 and HMGB1 were validated compared with the established markers S100B and LDH.

Here, we demonstrate by immuno-histochemistry on a melanoma tissue-microarray that RAGE protein expression is significantly upregulated in melanoma cells.

Serum concentrations of the candidate marker proteins sRAGE, S100A8/A9, and HMGB1 and of the known biomarkers S100B, and lactate dehydrogenase (LDH) were measured in 240 serum samples from melanoma patients (stage III = 170; stage IV = 70) using immunoassays (ELISA). sRAGE was identified as an independent marker of overall survival (OS) as decreased sRAGE serum levels correlate with poor survival and indicate progression ($P = 0.022$). Moreover, the RAGE ligands S100A8/A9 ($P < 0.000001$) and HMGB1 ($P < 0.000001$) are independent markers of progression-free survival (PFS). Most interestingly, patients that lacked S100B elevation despite progression could be identified by elevated S100A8/A9 or HMGB1 levels with a sensitivity of 42.9% and a specificity of 78.1% in contrast to LDH which fails in detecting those patients (sensitivity: 5.7%, specificity: 92.3%; $P < 0.000001$). Furthermore, using conditional inference trees the combination of S100B and S100A8/A9 identified 66% more patients with progressive disease than S100B alone.

In conclusion, we present three novel biomarkers for melanoma progression that are suitable as early markers of progression as well as independent markers of OS and that help to close the diagnostic gap of standard biomarker S100B. Thus, we shed light on RAGE signaling as a novel strong prognostic marker in melanoma as well as a promising target for anti-melanoma therapy.

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Senescent fibroblasts drive oncogenic properties in melanoma progression through CXCL6/GCP-2 induced CREB phosphorylation

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Ageing constitute the biggest risk factor for melanoma incidence and most likely its progression. The contribution of senescent fibroblast and their associated secretory phenotype (SASP) has been suggested to create a permissive microenvironment. However, the causal involvement of distinct SASP components and their downstream signaling cascade through transcription factors eventually driving melanoma progression are still poorly understood. We used a complementary approach with the analysis of SASP factors from conditioned medium of senescent fibroblasts in oncogenic assays *in vitro*, in murine models *in vivo* and correlated these finding with in skin sections from patients

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suffering from melanomas at different age groups. Here we have shown that CXCL6/GCP-2, a chemokine excessively released by senescent fibroblasts, *in vitro* and in skin sections of old individuals via the phosphorylation of the transcription factor CREB at serine 133, enhances the oncogenic properties like migration, extravasation and anchorage-independent growth of different melanoma cell lines both *in vitro* and in xenograft murine mouse model *in vivo*. This study provides insight into the role of what we believe to be a previously undescribed contribution of senescent fibroblasts in melanoma progression. Targeting senescent fibroblasts or CXCL6/GCP-2 may hold promise in the development of novel therapeutic strategies against melanoma progression.

P289

Canonical Wnt signalling enhances neural crest migration in zebrafish and epithelial mesenchymal transition associated melanoma cell invasion

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During embryonic development, Wnt family members and bone morphogenetic proteins (BMPs) cooperatively induce epithelial-mesenchymal transition (EMT) in the neural crest. Wnt and BMPs are reactivated during malignant transformation in melanoma. We previously reported that the BMP-antagonist noggin blocks EMT of melanoma cells in the neural crest of the chick embryo. Here we show that blocking the canonical Wnt signalling pathway using the β-catenin inhibitor PKF115-584 in the early zebrafish embryo disturbed neural crest migration leading to disorganized melanophore formation. In line, we show for the first time that canonical Wnt signalling is analogously important for EMT in human metastatic melanoma cells. Wnt3a reduced melanoma cell adhesion and enhanced migration, while PKF115-584 increased adhesion and reduced melanoma cell migration both *in vitro* and in the embryonic neural crest environment *in vivo*. These observations are accompanied by specific alterations in neural crest gene expression like inhibin, a TGF-β signalling modulator. This hints to a concerted action of Wnt and TGF-β/BMP signalling during EMT. Further we demonstrate that cytoplasmatic β-catenin expression increased during active migration/metastasis in a newly-established *in vivo* model for brain metastasis in the developing chick embryo. Together we present a novel role for Wnt3a in melanoma cell EMT/metastasis stressing the crucial role of embryonic neural crest signalling for the spreading of malignant melanoma.

P290

Increased angiotropism in the inflammatory microenvironment of melanomas acquired resistance to adoptive T cell therapy compared to untreated controls

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Tumor regression, remission and relapse after successful immunotherapy with adoptively transferred T-cells (ACT) targeting melanocytic antigens can be recapitulated in the genetically engineered Hgf-Cdk4 mouse melanoma model. Previously, we could show that primary Hgf-Cdk4 and transplantable HCmel3 melanomas can resist T-cell therapy (ACT) through inflammation-induced reversible dedifferentiation. Our data support a model of tumor progression and therapy resistance by melanoma cell plasticity induced by inflammatory signals in the microenvironment. It has been hypothesized that pericytic mimicry as a type of tumor cell plasticity support tumor progression by angiotropic melanoma cells with migratory, neural crest like properties. To test the hypothesis, that angiotropic growth of melanoma cells could be a mechanism how inflammation-induced plasticity contributes to melanoma progression and therapy resistance in our model, we analyzed ACT resistant and untreated melanomas histopathologically for signs of angiotropic growth. Interestingly, ACT resistant primary ($n = 10$) and HCmel3 transplantable ($n = 10$) melanomas revealed in 80% melanoma cells clearly cuff blood vessels at some distance (>1 mm) away from the main tumour mass. In contrast, only 20% of untreated primary or HCmel3 transplantable melanomas showed this phenomenon, originally described as angiotropism by histopathologist in human melanomas with worse prognosis. Whole genome mRNA expression analyses of control, relapsed HCmel3 melanomas and *in vitro* cultures of dedifferentiated HCmel3 relapse lines, identified a set of cell adhesion, migration and angiogenesis genes that is up-regulated in relapsed HCmel3 melanomas *in vivo*. These gene expression changes in HCmel3 relapse lines compared to parental HCmel3 melanoma cells can be largely recapitulated by short-term (72 h) TNF treatment. RT-PCR data confirmed the significant up-regulation of genes associated with vascular formation like vcam-1, icam-1, ang-2, pdgfrb in HCmel3 cells upon 72 h TNF exposure compared to untreated control. FACS-analyses identified the up-regulation of vcam-1 predominantly on dedifferentiated Ngfr(high) HCmel3 cells.

Taken together, these experimental results indicate that tumor relapse after initially successful T-cell immunotherapy involves an angiotropic phenotype switch of melanoma cells in the context of an inflammatory microenvironment. Further investigations are needed to investigate how inflammatory mediators can induces phenotypic melanoma cell plasticity by reactivation of lineage-specific progenitor programs which facilitate tumor progression and therapy resistance.

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Beta-catenin in the course of aquired resistance to the BRAFV600E inhibitor vemurafenib in malignant melanoma

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Many mechanisms are known for the development of resistance towards vemurafenib, the approved standard therapy for stage IV BRAFV600E melanomas. The majority of vemurafenib-treated melanoma patients rapidly develop therapy resistance within 7 months. A deep and accurate understanding of the underlying mechanisms is of great demand in order to overcome this fatal development. The importance of beta-catenin in these resistance mechanisms is unknown so far. Here, we examined the beta-catenin expression levels of resistant melanoma cells *in vitro* and *in vivo* and found increased beta-catenin protein levels in several resistant samples. Additionally, we found signs of nuclear translocation, a strong indicator for transcriptional activity of beta-catenin, and altered phosphorylation patterns. Therefore, we investigated the activity of the canonical Wnt signalling pathway via a luciferase reporter which surprisingly showed no increased luciferase signal in resistant cell lines compared to the sensitive parental cells. This prompted us to identify novel interaction partners of beta-catenin in vemurafenib resistant melanoma cells in order to elucidate the non-canonical, Wnt indepenent signalling cascades of beta-catenin in the resistant melanoma cells. We further invastigated the effects of resistance-mediating growth factors like hepatocyte growth factor or neuregulin-1 on beta-catenin modifications and signalling in order to gain hints for the mechanism behind the activation of beta-catenin. Functionally, knockdown of beta-catenin increased the effects of vemurafenib in two BRAFV600E melanoma cell lines via increased growth inhibition and apoptosis induction. In resistant cells, the knockdown partly re-sensitized the the cells to BRAF inhibition. Our results propose an important role of beta-catenin in the aquired resistance of melanomas towards vemurafenib.

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Role of Rac1 activation in melanoma progression

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Rac1 is a global player in control of proliferation, cell-cell-junctions and enzyme activity regulation. An activating Rac1 P29S mutation has been found to be present in 5% of UV-exposed melanoma. However the consequences of Rac1 activation in melanoma and downstream effectors are unclear. In this study we were looking for Rac1-GTP expression during melanoma expression and downstream effectors such as PAK1/2, HIF-1/2 and MEK/ERK *in vivo* and *vitro* and the consequences of blocking these pathways *in vitro*. We furthermore were identifiying clinical outcome of patients carrying a Rac1 P29S mutation in UV exposed melanoma. Finally we are correlating the infuence of Rac1 activation on cell-cell adhesion melanoma progression.

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Th2 cytokines from malignant cells suppress Th1 responses and enforce a global Th2 bias in leukemic cutaneous T-cell lymphoma

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In leukemic cutaneous T-cell lymphoma (L-CTCL), malignant T cells accumulate in the blood and give rise to widespread skin inflammation. Patients have intense pruritus, increased immunoglobulin E (IgE), and decreased T-helper (Th)-1 responses, and most die from infection. Depleting malignant T cells while preserving normal immunity is a clinical challenge. L-CTCL has been variably described as a malignancy of regulatory, Th2 and Th17 cells.

We analyzed phenotype and cytokine production in malignant and benign L-CTCL T cells, characterized the effects of malignant T cells on healthy T cells, and studied the immunomodulatory effects of treatment modalities in patients with L-CTCL.

Twelve out of 12 patients with L-CTCL overproduced Th2 cytokines. Remaining benign T cells were also strongly Th2 biased, suggesting a global Th2 skewing of the T-cell repertoire. Culture of benign T cells away from the malignant clone reduced Th2 and enhanced Th1 responses, but separate culture had no effect on malignant T cells. Coculture of healthy T cells with L-CTCL T cells reduced IFN production and neutralizing antibodies to interleukin (IL)-4 and IL-13 restored Th1 responses. In patients, enhanced Th1 responses were observed following a variety of treatment modalities that reduced malignant T-cell burden.

A global Th2 bias exists in both benign and malignant T cells in L-CTCL and may underlie the infectious susceptibility of patients. Th2 cytokines from malignant cells strongly inhibited Th1 responses. Our results suggest that therapies that inhibit Th2 cytokine activity, by virtue of their ability to improve Th1 responses, may have the potential to enhance both anticancer and antipathogen responses.

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Stigmatisation, avoidance behaviour and difficulties in coping are common among adult patients with vitiligo

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Vitiligo is a non-contagious, acquired depigmentation disorder, characterised by loss of the inherited skin colour. The disease is often accompanied by an impaired well-being.

The aim of this study was to explore quality of life (QoL), coping, depression and stigmatisation in a controlled, cross-sectional study utilising the Dermatology Life Quality Index (DLQI), the Adjustment to Chronic Skin Disorders Questionnaire (ASC) and the Beck Depression Inventory (BDI). In addition some disease-related questions were included. The study included 96 patients (35 males/61 females, mean age 41.7 years, mostly skin phototypes I–III, mean disease duration 17.4 years and mean age of onset 24.1 years) and 23 age, gender and skin phototype matched healthy controls. The results revealed a positive family history in 27.1%. Stigmatisation was very common. 90% had frequently been asked questions about their appearance, 24% had been subject to nasty comments. Almost two thirds believed that psychological stress influenced their vitiligo. The same percentage avoided situations because of their vitiligo or concealed their white spots in presence of other people. Patients' mean questionnaires' scores were significantly higher compared to controls (DLQI 4.9 vs. 1.6; ASC-social anxiety/avoidance 36.9 vs. 22.1; ASC-helplessness 27.3 vs. 16.0; ASC-anxious-depressive mood 19.4 vs. 15.6), except for the BDI (6.8 vs. 7.3). Notably, patients who had never experienced low-level stigmatisation, scored highest in DLQI and social anxiety/avoidance. High-level stigmatisation had a different effect. Avoidance behaviour and concealing vitiligo correlated with all questionnaires' scores. QoL was only little affected in two thirds, three quarters were not depressed. Treatment with narrow-band activated pro-pseudocatalase PC-KUS led to reduction of social anxiety, anxious-depressive mood and depression.

Taken together, vitiligo can worsen QoL in some patients and can make them more socially anxious, helpless and anxious-depressive. Hence, after individual evaluation, psychological counselling should be considered for patient care in this disease.

Miscellaneous

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Comparison of the adhesion disposition of conventional and modern wound dressings *in vitro*

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Objective: Wound dressings that adhere to the wound surface can disrupt the wound bed and destroy newly formed, healthy tissue on removal, resulting in a disturbed, rough surface. This often happens with simple gauze pad. To avoid conglutination with the wound, e.g. combined fleece compresses possess a micro-porous polyester foil or impregnated gauze is used. So far, it hasn't been studied whether the conglutination proclivity of conventional dressings is different from modern wound dressings, e.g. foams featuring a WCL (wound contact layer). Hence, we have evaluated the adhesion disposition of conventional and modern wound dressings *in vitro*.

Method: For the conglutination tests, simple cotton gauze (A: cotton gauze pads, Fuhrmann) was chosen as positive control. Four combined fleece compresses (B: Solvaline®N, Lohmann & Rauscher; C: Solvaline®N *neu*, Lohmann & Rauscher; D: Melolin®, Smith & Nephew; E: Askina® Pad, B.Braun) and two impregnated gauzes (F: Lomatuell® H, Lohmann & Rauscher; G: Clauden®, Lohmann & Rauscher) as well as four modern foam dressings with WCL (H: Suprasorb®P+WCL, Lohmann & Rauscher; I: Allevyn gentle, Smith & Nephew; J: Mepilex®border, Mölnlycke Health Care; K: Biatain®, Coloplast) were picked for analysis. A fibrinogen/thrombin layer was applied onto the tissue substitute (10% (w/v) gelatine, 10% (w/v) milk powder) on which the dressing samples (3 cm x 4 cm) were put. Evaluation of the adhesion disposition was carried out by measurement of the force necessary to remove the dressing from the tissue substitute.

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Results: It could be shown that by combination of a fleece compress with a micro-porous polyester foil the adhesion disposition can be significantly reduced compared to a simple cotton gauze ($P < 0.001$). Distinctly less force was needed to remove the dressings B–E from the tissue substitute. The impregnated gauzes F and G did not exhibit any conglutination in the test. All modern wound dressings, except dressing J, demonstrated a significantly reduced adhesion *in vitro* compared to cotton gauze. Dressing J possess an adhesive contact layer, hence, a distinctly stronger force was needed to remove the samples from the tissue substitute.
Conclusion: With the help of an *in vitro* tissue model, the adhesion disposition of wound dressings could be quantified and evaluated. It could be shown that conventional dressings are capable to exhibit a comparable low conglutination with the wound as modern wound dressings.

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Analysis of the fluid management by hydroactive wound dressings with the help of an *in vitro* maceration model

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Objective: Modern wound dressings are expected to maintain a humid wound milieu without allowing maceration at the wound edges. Such hydroactive dressings mainly consist of alginate or contain sodium carboxymethylcellulose. Both polymers form fibres that can be processed to fleece compresses or tamponade strips and exhibit a high fluid uptake. However, highly exuding wounds may lead to macerated wound edges. Moreover, during gel formation loss of shape can be observed which results in reduced wound coverage. Hence, the fluid management of hydroactive dressings was analysed using a special maceration model.
Method: For the tests, two dressings with sodium carboxymethylcellulose (A: Aquacel®, ConvaTec; B: Aquacel® Extra, ConvaTec), one dressing consisting of cellulose/ethyl-sulfonate-cellulose (C: Suprasorb® Liquacel, Lohmann & Rauscher), and a calcium alginate tamponade (D: Suprasorb® A Tamponade, Lohmann & Rauscher) were used. They were applied to an artificial wound in the tissue substitute (10% (w/v) gelatine, 10% (w/v) milk powder) for the maceration test. The evaluation of fluid uptake and distribution in the dressings was performed by video recording. In addition, the shape loss of the dressings, the maximal fluid uptake and the time to maceration was determined.
Results: It could be shown that the sodium carboxymethylcellulose dressings exhibit a distinct shrinkage during fluid uptake with app. 29 and 36% for dressings A and B, respectively. Dressing C showed with only 18% shrinkage significantly higher form stability. For dressing D no loss of surface coverage was observed. However, D demonstrated with just 20 ml fluid uptake the lowest fluid holding capacity. A similar fluid uptake till break point of maceration was found for dressing A. Dressings B and C exhibited significantly higher values with app. 25 and 30 ml, respectively. Moreover, with A and B maceration already occurred before the dressings were completely soaked. Leakage with dressings C and D was only observed after they were completeld gelled.
Conclusion: An *in vitro* maceration model was successfully used to quantify and evaluate the differences between hydroactive wound dressings. This model is hence suitable to analyse the fluid management in an *in vivo* like situation *in vitro*.

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In vitro evaluation of the fluid distribution in different wound dressings during negative pressure wound therapy (NPWT)

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Objective: NPWT is clinically effective in the treatment of chronic-stagnating wounds. Studies suggest that the positive effects result from cell recruitments to the wound site, where they contribute to granulation tissue formation. We showed that dressings used for NPWT exhibit different effects on the cells, they especially grow into large-pored foams, and on the wound area surface and the surrounding tissue. Here, we look at the differences in fluid distribution during NPWT using a large-pored PU-foam dressing (*Suprasorb® CNP foam/Lohmann & Rauscher), a drainage foil (+Suprasorb® CNP drainage foil/Lohmann & Rauscher) and specialised NPT-dressing system (#KCI ABThera System/KCI).
Method: Dressings were placed on the tissue model (10%gelatine, 10%milk powder) and connected to a vacuum pump (Suprasorb® CNP-P1/Lohmann & Rauscher) by a vacuum seal. Experiments were carried out at −120 mmHg for 8 h. The dispersal of the fluid under the dressings was tracked by sensitive IR imaging. For this, the liquid was cooled to a temperature of 4°C in the fluid supply while the ambient temperature was held constant at 22°C.
Results: The dressings tested exhibited a quick fluid distribution in the first 60 min with slopes reaching from 0.97 to 1.31. Steady states were reached after app. 160 min. However, only for the PU-foam* and the drainage foil+ a complete and uniform spread of the fluid was observed, while underneath the NPT-dressing system# a fluid distribution of no more than 70% was achieved.
Conclusion: NPWT produces heterogeneous pressures at the wound ground, leading to gradients that control the drainage of interstitial fluid. Thus, it is of interest to investigate the fluid distribution in different dressings during NPWT. In this study, using a gelatine-based tissue model, it could be shown that the fluid distribution during NPWT differs among dressings.

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In vitro evaluation of the influence of the pH on the antimicrobial activity of PHMB- and silver-functionalized wound dressings

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Objective: There is a shift towards higher pH-values in chronic compared to acute wounds ('alkaline shift'). The pH in chronic wounds most commonly has a range of 6.5 to 8.5. This alkalization is due to tissue necrosis and presence of microorganisms. Hence, it is of interest to determine the pH influence on the efficacy of antimicrobial wound dressings. We have used two experimental systems one based on the agar diffusion test (ADT) and the other on microplate-laser-nephelometry (MLN) to evaluate the pH influence on the activity of PHMB- and silver-functionalized dressings against *S. aureus* and *P. aeruginosa*.
Method: ADT was performed according to DIN58940-3 with samples of 0.6 cm^2. The zones of inhibition (ZOI) were measured to evaluate the antimicrobial efficacy at different pH (5.0, 6.0, 7.0, 8.0, and 9.0). For MLN, dressing extracts were prepared corresponding to DIN10993-12 at the respective pH (6.0, 7.0, 8.0, and 9.0). Bacterial growth was measured according by solution turbidity, dose-response-curves were determined and IC50 concentrations were calculated. The IC50 was used to evaluate the antimicrobial efficacy at different pH values.
Results: Low pH (5.0) effectively inhibited microbial growth in solution but exhibited no significant effect in the ADT. PHMB- and silver-dressings showed antibacterial activity in all tests. Yet, total effectiveness varied at different pH *in vitro*, e.g. the efficacy of the PHMB-containing dressing increased significantly with increasing pH. Moreover, the dressings themselves influenced the local pH; either adjusting the effective pH to higher values or reducing it to a more favorable milieu for wound healing.
Conclusion: Employing both, the agar diffusion test and microplate-laser-nephelometry it could be shown that PHMB- and silver-functionalized dressings possess a pH-dependent antimicrobial activity. Moreover, it was found that the influence of the pH on the efficacy of the dressings is slightly different for *S. aureus* and *P. aeruginosa*.

P299

Omalizumab does not inhibit mast cell and basophil activation *in vitro*

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The mechanism of action of omalizumab (OMA), which is currently licensed for the use in severe allergic asthma, is thought to be the reduction of free IgE serum levels and the subsequent down-regulation of FcεRI on mast cells (MC) and basophils. It has recently been demonstrated that OMA is highly effective in chronic spontaneous urticaria (CSU). While increased levels of IgE can be found in some patients with CSU, different or additional mechanisms than neutralization of free IgE are thought to be reponsible for the effects of OMA in CSU. Since OMA cannot bind to receptor-bound IgE, we hypothesized that OMA can form cell-stabilizing immune complexes after contact with serum from CSU patients resulting in the immediate reduction or even blockade of MC and/or basophil activation, i.e. by cross-activation of inhibitory Fcγ receptors. To test this, serum from CSU patients (auto-reactive or not) or healthy subjects was pre-incubated with OMA to allow for the formation of immune complexes. Subsequently, freshly isolated human skin MC or basophils were co-incubated with serum/OMA, cells were activated and the release of histamine was determined. Auto-reactive and non auto-reactive sera from CSU patients and sera from healthy subjects induced low to moderate histamine release, while anti-IgE stimulation resulted in extensive degranulation. Notably, pre-incubation of sera with OMA did neither reduce serum-induced nor FcεRI-mediated histamine release. In contrast, co-incubation of human skin MC with the DARPin-Fc fusion protein as positive control for negative signaling via FcεRI-FcγRIIb cross-activation reduced the anti-IgE-induced histamine release by 50%. In summary, our results suggest that the OMA therapy-induced rapid reduction of CSU symptoms results from the elimination of an activating signal rather than the generation of a negative, inhibitory signal. Further *in vivo* and *ex vivo* tests need to be carried out to identify the mechanism of action of OMA in CSU.

P300

Advanced *in vitro* testing for dermal drug delivery – novel approaches for simulating and analyzing human wounds

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At present, clinicians still face severe medical problems associated with the impaired healing ability of chronic wounds. In addition, colonization of these wounds by resistant pathogens further impedes the healing process. According to demographic trends, a constantly aging society will lead to a rising number of patients suffering from chronic wounds. For rational development of novel therapeutic systems for effective treatment of these wounds, appropriate models are mandatory for reproducible *in vitro* testing of novel systems. In addition, conventional wound assessment techniques for evaluating wound size and depth of such *in vitro* models are destructive and lacking accuracy. Considering these facts, there is a strong need for a valid *in vitro* model simulating chronic wounds and novel technologies for precise wound characterization.
We here demonstrate a human *in vitro* wound model characterized by a linear, rectangular wound geometry, tilted wound edges and completely surrounded by intact skin to overcome limitations of existing wound models and to simulate typical characteristics of human wounds. In particular, we could show that linear wound models overcome the problem of varying wound gap sizes of standard circular wounds created by punch biopsies. With respect to their three-dimensional geometry and reproducibility, we could show that linear wounds do not vary in their wound size depending on the sectional plane and reveal a uniform consistent appearance along the entire length of the wound bed. In addition, for adequate simulation of the human *in vivo* situation, we established a wounding technique which enables a reproducible preparing of tilted wound edges and restriction of the wound bed to all sides of the wound in order to have a wound bed completely surrounded by intact skin.
Furthermore, we here introduce a novel technical approach for a more realistic and accurate documentation of three-dimensional wound appearance. For that purpose, we use optical profilometry, a technique based on the principle of white light reflection. The topography images successfully reflect the structured surface, wound edges and the wound bed with high accuracy. Moreover, we could demonstrate the applicability of optical profilometry for virtual sectioning of the wound samples in order to evaluate the wound geometry without destruction of the tissue and analysis along the lateral and vertical axis.
Our findings indicate the suitability of the *in vitro* model for adequately mimicking human wounds with the potential to study the wound healing potential of novel therapeutics. Further, we demonstrate the feasibility of optical profilometry for non-invasive three-dimensional wound characterization overcoming limitations of conventional histological analysis methods.

P301

Plasma application in human skin – molecular analyses *in situ*

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The development of plasma sources producing tissue tolerable, i.e. non-thermal atmospheric pressure plasmas, opens a number of new applications. This attracts growing interest especially in dermatological research, for example in the field of wound healing and skin cancer therapy. To date there are a number of investigations concerning the effect of plasma on different cell types *in vitro*. The results point to dose dependent effects. At low doses plasma exposure stimulates cell growth and proliferation while at higher exposure times it can cause apoptosis. This has been demonstrated on human HaCaT keratinocytes as well as on T helper cells (THP-1) (Barton et al, 2013; Bundscherer et al, 2013). Both cell types are essential for skin homeostasis and wound healing. In order to apply plasma on patient skin it is crucial to tailor plasma to achieve only the desired effects and minimise side effects. Based on our earlier findings on DNA microarray and mass spectrometry for protein identification we found a group of promising proteins and signalling molecules, i.e. response to oxidative stress and wound healing related molecules.
The aim of this study was to transfer the finding of our *in vitro* data into 3-dimensional skin tissue. Therefore human skin was exposed to plasma generated by a plasma jet (kinpen MED; Neoplas, Greifswald). Thereafter skin samples were processed for histological analyses and immunofluorescence. Besides general histology (H&E staining), samples were examined for DNA strand breaks (gamma H2A.X), proliferation (Ki67) and apoptosis (TUNEL) as well as differentiation pattern (keratin 14 and keratin 1).
The results indicate that after 1 min local treatment the epidermal homeostasis was not impaired. Expression of keratin 1 and keratin 14 remained comparable to untreated skin samples. In addition, no increased apoptosis (evaluated by TUNEL staining) was detected, although gamma H2A.X stained positive in plasma treated skin samples. Strikingly, in a number of samples stimulation of proliferation of keratinocytes was detected by Ki67 immunoreactivity.
Under the applied conditions the skin remained intact and holds the capacity for DNA repair since no induction of apoptosis was detected. Instead a temporary stimulation of proliferation was induced by local plasma treatment for 1 minute. However, the underlying mechanisms and responsible molecules are currently under investigation.
Our data show for the first time the impact of plasma on the human skin in situ and might bring forward the application of plasma in dermatology.

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Literature: Bundscherer, L., K. Wende, et al. (2013). "Impact of non-thermal plasma treatment on MAPK signaling pathways of human immune cell lines." Immunobiology.
Barton, A., Bundscherer, L., et al. (2012) " Impact of cold atmospheric pressure plasma on human skin cell-lines." 4th International Conference on Plasma Medicine, Orlans, France.

P302

Changes in lipid composition potentially modify PPARalpha signaling in a model of chronic skin barrier impairment

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In humans, loss of function mutations in the profilaggrin gene cause ichtyosis vulgaris(IV), and are associated with disease formation in atopic dermatitis(AD). Due to a missense mutation in the profilaggrin gene, flaky tail mice lack functional filaggrin protein. PPARs play a prominent role in cutaneous homeostasis by controlling epidermal lipid synthesis, keratinocyte differentiation and skin inflammation.

In this study, we aim to dissect the role of PPAR signaling in the skin of flaky tail mice. We assessed the expression of PPARalpha, PPARbeta/delta, PPARgamma, several upstream and downstream signals and cutaneous lipid composition in the skin of flaky tail mice. Our murine model revealed a reduction in PPARalpha mRNA expression levels whereas PPARbeta/delta and PPARgamma levels were not altered. This corresponds with a decrease in lipid metabolites including leukotrienes and eicosanoids specifically involved in PPARalpha signaling. Together these data demonstrate alterations in skin lipid metabolism in flaky tail mice and decreased PPARalpha signaling.

P303

Investigation on the influence of skin acidification on ammonia molecules leaving the skin surface via diffusion – an exploratory study

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In contrast to other gaseous molecules the implications of ammonia leaving the skin surface via diffusion for skin investigation are only little evaluated. Due to its gaseous properties and its strong relation to the pH of the chemical environment ammonia might serve as parameter for skin barrier function as well as for the investigation of ammonia producing mechanisms in the skin. The aim of the present study is to assess ammonia leaving the skin surface via diffusion along with skin surface pH (pH) before and after glycolic acid induced acidification of the skin.

Overall, 12 women and 12 men were included into the study after obtaining informed written consent. The site of investigation was the medium part of the non-dominant forearm (FA). Assessments were performed before and 20 min after application of an acidifying lotion. For collection of ammonia leaving the skin surface the volunteers placed their FA for 2 min on a petri dish filled with 5 ml of ion free water without direct contact between skin and water. Afterwards, quantification of ammonia was performed spectrophotometrically. The pH was assessed using a glass electrode that is commercially available. The acidifying preparation contained 10 percent glycolic acid with a pH of 3.8 and was commercially available, too. For statistical evaluation average values and standard deviations were calculated and before and after comparisons as well as correlation/regression analyses were performed.

The amounts of ammonia leaving the skin surface decreased significantly from 2,421,10 ng/cmmin before acidification to 1,140,67 ng/cm min after acidification of the skin ($P < 0.001$), while the pH decreased significantly from 5,080,37 to 4,010,08 ($P < 0.001$). The exploratory correlation analysis revealed a significant negative correlation between ammonia and pH before acidification ($r = -0.643$; $P = 0.001$) which reversed to a positive correlation after acidification ($r = 0.510$; $P = 0.015$). A regression analysis of the values from before and after acidification together with respect to different types of relationships between ammonia and pH revealed a linear relationship ($R = 0.130$; $P = 0.016$) which was by far exceeded by a quadratic relationship with a maximum around a pH of 4.75 ($R = 0.570$; $P < 0.001$, exact $P = 3 \times 10^{-8}$).

The results obtained in the present study show that application of the glycolic acid containing preparation had impact on all parameters assessed. While the decrease of ammonia leaving the skin surface after acidification might be explained chemically with a trapping of ammonia by hydrogen ions, the quadratic relationship reminds of pH activity profiles of enzymes known in biochemistry. Therefore, the question arises, whether the glycolic acid induced decrease of skin pH had influence on ammonia releasing enzymatic mechanisms within the stratum corneum. Both direct effects on enzymes such as transglutaminases or histidine ammonia lyase and indirect effects for example via cathepsins which are known to have high activity at acid pH values as well as regulatory function on enzymes such as transglutaminases appear possible. Further studies are required to assess the relation between ammonia molecules leaving the skin surface and such enzymes.

P304

Function and regulation of inter-α-trypsin inhibitor heavy chain 5 (ITIH5) in normal human skin, inflammatory skin diseases, constitutive knock-out mice and murine 3D-skin models

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Inter-α-trypsin inhibitors are protease inhibitors which consist of one light chain (bikunin) and different heavy chains (ITIHs). There have been many studies of the ITI molecules, proposing an involvement in various acute-phase processes. The only function known so far of the ITI heavy chains is the covalent linkage to hyaluronic acid (HA). Inter-α-trypsin inhibitor heavy chain 5 (ITIH5) is a recently characterized novel member of the ITIH gene family. Since there is virtually no knowledge on the distribution and function of ITIH proteins in skin tissue, we performed a systematic characterization of ITIH expression in healthy and diseased skin. Using GeneChip® expression profiling we found that ITIH5 represents the major ITIH family member expressed in human skin. Moreover, the use of quantitative reverse transcription PCR and an ITIH5-specific antibody indicated that ITIH5 is predominantly produced by dermal fibroblasts. Immunohistochemical analysis revealed a clearly detectable ITIH5 protein expression in normal skin. Interestingly, ITIH5 expression was significantly upregulated in inflammatory skin diseases, i.e. in the suprabasal layers of patients with psoriasis, atopic dermatitis and allergic contact dermatitis. Furthermore 3D skin models employing murine ITIH5−/− epidermal keratinocytes and dermal fibroblasts as well as skin specimen from various skin areas of ITIH5−/− mice revealed a significantly altered epidermal structure compared to wildtype controls. ITIH5 may constitute a novel regulatory molecule of the human skin that could play an important role in inflammation via its interaction with HA.

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