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ALLERGY

P001 (OP02/03) | Allergy-inducing chromium compounds trigger potent innate immune stimulation via ROS-dependent inflammasome activation

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Chromium allergy is a common occupational skin disease mediated by chromium (VI)-specific T cells that induce delayed-type hypersensitivity in sensitized individuals. Additionally, chromium (VI) can act as irritant. Both responses critically require innate immune activation, but if and how chromium (VI) elicits this signal is currently unclear.
Using human monocytes, primary human keratinocytes and murine dendritic cells we show that chromium (VI) compounds fail to trigger direct pro-inflammatory activation but potently induce processing and secretion of IL-1β. IL-1β release required priming by phorbol-ester or Toll-like receptor stimulation and was prevented by inhibition of K+ efflux, NLRP3 depletion or Caspase-1 inhibition, identifying chromium (VI) as novel hapten activator of the NLRP3 inflammasome. Inflammasome activation was initiated by mitochondrial ROS production triggered by chromium (VI) as indicated by sensitivity to treatment with the ROS scavenger N-acetyl cysteine and a coinciding failure of K+ efflux, Caspase-1 or NLRP3 inhibition to prevent mitochondrial ROS accumulation. IL-1β release further correlated with cytotoxicity that was secondary to ROS, K+ efflux and NLRP3 activation. Intriguingly, trivalent chromium was unable to induce mitochondrial ROS production, inflammasome activation and cytotoxicity, suggesting that oxidation state-specific differences in mitochondrial reactivity may determine inflammasome activation and allergic/irritant capacity of different chromium compounds.

P002 | Potent NLRP3 inflammasome activation by the allergy-inducing HIV reverse transcriptase inhibitor abacavir

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Drug allergies are adverse drug reactions with immunological origin. Delayed-type drug allergies commonly manifest as a severe cutaneous drug reactions induced by drug-specific T cells. Generation of such drug-reactive T cells likely requires innate immune stimulation but it is currently unclear if and how drug allergens elicit such a response. Here we analysed whether abacavir, an HIV-1 reverse transcriptase inhibitor inducing severe delayed-type drug hypersensitivity, can trigger innate immune activation that may contribute to its allergic potential. We show that abacavir fails to generate direct innate immune activation in human monocytes but potently triggers IL-1β release upon pro-inflammatory priming with phorbol ester or Toll-like receptor 8 stimulation. IL-1β processing and secretion was sensitive to Caspase-1 inhibition, NLRP3 knockdown and K+ efflux inhibition and was not observed with other non-allergenic nucleoside reverse transcriptase inhibitors identifying abacavir as specific inflammasome activator. It further correlated with dose-dependent mitochondrial ROS production and cytotoxicity indicating that inflammasome activation resulted from mitochondrial damage. However, both NLRP3 depletion and inhibition of K+ efflux mitigated abacavir-induced mitochondrial ROS production and cytotoxicity suggesting that these processes were secondary to NLRP3 activation. Our data identify abacavir as inflammasome-stimulating drug allergen. They implicate a potential contribution of innate immune activation to medication-induced delayed-type hypersensitivity, which may stimulate novel concepts for treatment and prevention of drug allergies.

P003 (OP01/02) | Allergic contact dermatitis is controlled by microbiota via a TLR-2 dependent mechanism

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Microbiota play a pivotal role in the development and calibration of host immunity. A variety of bacterial entities are populating the inner and outer surfaces of our body which are composed of pathological or commensal bacteria. Allergic disorders are increasing worldwide and the influence of microbial exposition has been intensively discussed in this context. However, the effect of the microbiome on the development of the allergic contact dermatitis as one of the most frequent occupational dermatological disorders has not been evaluated so far. In our study, we analyzed the experimental model of the contact hypersensitivity (CHS) reaction, a CD8+ Tc1-mediated cutaneous inflammation which resembles the allergic contact dermatitis in men. We performed experiments with germ-free mice (GF) which revealed that the cutaneous inflammation (inhibited ear swelling and reduced cellular infiltration) were significantly reduced compared to control mice. Re-colonized GF mice showed an unaffected CHS reaction, excluding a general defect in the immune

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response under germ-free conditions. Interestingly, the resulting hapten-specific Tc1 cell responses (T cell proliferation, IFN-γ production) was unaffected in sensitized GF animals, but we found increased amounts of IL-10 and IL-17 compared to controls. Next, we treated mice with a regime of antibiotics that predominantly eliminates the gut but not the cutaneous microbiota. Unexpectedly, eradication of the intestinal microbiota completely mimicked the GF phenotype with regard to the clinical symptoms and T cell response, indicating that gut- but not the skin-associated pathogens control the CHS reaction. As TLR2-mediated immune responses have been discussed to be involved in cutaneous inflammation and pathogen-induced immune tolerance, we aimed to address GF and antibiotics-treated TLR2 deficient mice in the CHS model as well. In the absence of TLR2-mediated signaling, the allergic cutaneous inflammation was unaffected, revealing that TLR2 signaling is critically involved in the control of the CHS reaction by the gut microbiome. We furthermore confirmed these results by repeated injection of the TLR2 agonists Pam2Cys and Pam3Cys which also resulted in significantly abrogated CHS symptoms. Conclusively, the cutaneous inflammation (CHS) in response to contact allergens is controlled by the intestinal- but not the skin-related microbiome via TLR2-mediated mechanisms.

P004 | Diabetes mellitus type I protects from the development of an allergic contact dermatitis in mice

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Epidemiological studies noted a lower prevalence of allergic contact dermatitis (ACD) in individuals with autoimmune diseases like type I diabetes or rheumatoid arthritis as compared to healthy individuals. However, the effect of autoimmune mechanisms on the development of the ACD as a hapten-specific cutaneous inflammatory disorder has not been investigated up to now. In our study, we used non-obese diabetes (NOD) mice which spontaneously develop an autoimmune insulin-dependent diabetes mellitus, mimicking the diabetes type I in men and the murine model of the contact hypersensitivity reaction (CHS), a CD8+ Tc1-mediated cutaneous inflammation which resembles the ACD. Female NOD mice were considered diabetic when blood glucose levels showed two consecutive readings above 250 mg/dL. In order to induce a CHS, the mice were epicutaneously sensitized with a contact sensitizer (eg, the hapten TNCB), followed by an application of the hapten onto the ear to elicit the CD8+ Tc1-mediated skin inflammation. We compared the impact of the diabetic phenotype on the development of the CHS reaction in diabetic vs. non-diabetic NOD mice and also non-obese resistant (NOR) mice as a further non-diabetic control strain. 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 and reduced inflammatory infiltrate) as compared to non-diabetic NOD and NOR mice. In contrast, we did not observe an impaired hapten-specific T cell response (T cell proliferation, Tc1-cytokine (IFN-γ, IL-2) production) in skin-draining lymph nodes or the spleen of diabetic mice. However, increased levels of IL-10 were detected in diabetic mice with reduced CHS reactions as compared to non-diabetic animals with CHS development. In conclusion, our data indicate that the manifestation of an autoimmune disease like diabetes mellitus type I circumvents the magnitude of an allergic CD8+ Tc1-mediated skin inflammation 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 a novel link between the development of allergic and autoimmune diseases may result in new preventive strategies for inflammatory disorders.

P005 | In chronic spontaneous urticaria, high numbers of dermal endothelial cells, but not mast cells, are linked to recurrent angioedema

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Background: Chronic spontaneous urticaria (CSU) is an inflammatory skin disorder characterized by recurrent wheals, angioedema, or both. Recent studies showed that the number of endothelial cells is increased in the skin of CSU patients, but the underlying mechanisms and clinical implications of this are unclear.

Aim: To evaluate whether mast cell or endothelial cell numbers in CSU patients correlate and whether they are relevant for disease duration, disease activity or the presence of clinical features.

Methods: We determined the numbers of CD31+ endothelial cells and mast cells in non-lesional skin of 30 CSU patients using quantitative histomorphometry and assessed their correlation with each other as well as with clinical features including disease duration, disease activity, and the occurrence of angioedema.

Results: The numbers of endothelial cells and mast cells were high in the non-lesional skin of CSU patients, but did not correlate. Both, endothelial cell and mast cell numbers did not correlate with the duration or the activity of disease. Interestingly, patients with high numbers of cutaneous CD31+ endothelial cells had higher rates of recurrent angioedema and vice versa.

Conclusions: Based on these findings, we speculate that vascular remodeling and mast cell hyperplasia in CSU patients occurs independently and due to different mechanisms. Targeting of the mechanisms that drive neoangiogenesis in CSU may result in novel therapeutic strategies for the management of patients with angioedema.

P006 | IgE directed against *S. aureus* superantigens are more frequent in chronic spontaneous urticaria patients

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Chronic spontaneous urticaria (CSU) is a frequent disorder that presents with recurrent itchy wheal and flare-type skin reactions and/or angioedema. Emerging data and the successful introduction of anti-IgE therapy (omalizumab) in CSU suggests a central role of IgE in the pathophysiology of this disease. Which antigens are detected by IgE in CSU patients is unknown, as most patients do not show specific IgE towards "classical" environmental allergens. Here, we investigated the expression and basophil activating effects of IgE directed towards Staphylococcus aureus superantigens (SAS) (Staphylococcal enterotoxin mix: SE; Staphylococcal enterotoxin B: SEB) in CSU patients and healthy controls, in an ELISA-based approach and via basophil histamine release assay, respectively. Half (51%) of the 49 CSU patients compared to 33.3% of 15 healthy controls analysed had detectable levels of IgE-anti-SE by ELISA. Mean IgE-anti-SE serum levels were significantly higher in CSU patients (0.23 IU 0.08) as compared to healthy controls (0.16 IU 0.08; P=.04). IgE-anti-SE serum levels in CSU patients correlated significantly with total IgE levels (Rs=0.52. P<.001). In 15 CSU patients IgE-anti-SEB serum levels were also significantly higher (0.28 IU 0.09) as compared to healthy controls (0.06 IU 0.08; P=.03); and they also correlated with total IgE levels (Rs=0.54 P=.04). Histamine release in response to SEB as antigen was slightly but significantly higher in basophils loaded with the serum of CSU patients (mean: 6%, range: 23-0%) as compared to basophils loaded with the serum of healthy controls (mean 3%, range 7-0%, P<.05). Clinically, IgE-anti-SEB-positive patients were characterized by significantly longer duration of the disease (Rs=0.51, P=.05). In summary, the occurrence of functional IgE-anti-SAS is significantly increased in CSU patients and linked to a prolonged course of CSU. Our results encourage the further assessment of these type of IgE antibodies and their role and relevance in the pathophysiology of CSU.

P007 | Metabolic activation of prohaptens in a modified KeratinoSens assay using rat liver S9 (cytosolic) fraction spiked with cutaneous cytochrome P450s

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Skin is a uniquely susceptible target organ for allergic contact dermatitis to environmentally encountered small molecular weight compounds (haptens) that can form antigens. The prevailing mechanistic explanation for these types of allergic responses requires some type of chemical activation that drives binding of the hapten or prohapten to macromolecules such as proteins or peptides to form an antigen. Some haptens are sufficiently reactive to bind directly but other less reactive prohaptens require metabolic activation in the skin to haptens. As an approach to reducing the need for experimental animals one has attempted to develop nonanimal alternatives an example of which is the KeratinoSens assay. However, the identification of prohaptens in these assays has proven to be challenging because of inconsistent prohapten bioactivation. Preincubation or coincubation of the prohapten with single cytochrome P450 isoenzyme (CYP) cocktails or CYP-containing microsomes are generally very cytotoxic. Accordingly the use of the cytosolic S9 fraction prepared from rat liver has been tried (S9). One limitation of this approach is that rat liver S9 does not contain human skin CYPs that might be capable of activating these prohaptens. Carvoxime is a potent prohapten known to be a strong sensitizer in mammalian skin and in the murine LLNA and is activated by human skin CYP 1B1 that is not expressed in liver and was not a sensitizer in the KeratinoSens assay using rat liver S9. This led us to redesign our protocol by spiking rat liver S9 with human cutaneous CYPs including CYP 1B1 and measuring the activation of NRF2 in KeratinoSens by the luciferase assay and the cytotoxicity by the MTT assay. Cinnamic aldehyde served as a positive control since it does not require metabolic activation and SDS was used as an irritant control. Addition of carvoxime to the spiked preparation as well as CYP 1B1 itself strongly activated NRF2 and heat inactivated preparations showed no reactivity. These results confirm that carvoxime is specifically activated by CYP. Spiking of rat liver S9 with cutaneous CYPs increases the sensitivity of the KeratinoSens assay in identifying prohaptens specifically activated by cutaneous CYP isoenzymes.

P008 | Tissue factor inhibition and protease-activated receptor two mutation protect from contact hypersensitivity

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Tissue factor (TF) and protease-activated receptor 2 (PAR2) regulate hemostasis, thrombosis and cardiovascular function. They also act in (cutaneous) inflammation, but their detailed interaction with the innate and adaptive immune system is poorly understood. PAR2 is activated by a broad array of serine proteases. The binary complex of TF and activated coagulation factor VII (VIIa) and the ternary coagulation initiation complex TF-VIIa-Xa cleave and activate PAR2. If the proteolytical cleavage site of PAR2 is defective, thrombin-activated protease-activated receptor 1 (PAR1) transactivates PAR2. Here, we investigated the role of TF and PAR2 signaling in contact hypersensitivity (CHS) as a murine model for allergic contact dermatitis in humans. Functionally active TF was blocked by an anti-TF antibody in C57BL/6 wild-type mice. Also, two different PAR2 mutant mouse strains were generated: The first PAR2 mutant (R38E) is insensitive to proteolytic activation and only allows the thrombin-induced PAR1-mediated transactivation. The second mutant (G37I) is resistant to the proteolytic effect of coagulation factor Xa without impairing the activation by other proteases. We show that TF blockade in C57BL/6 wild-type mice and dysfunctional PAR2 signaling in both transgenic mouse strains led to an impaired 2,4,6-trinitrochlorobenzene

(TNCB)-induced CHS reaction. The cutaneous inflammatory response of CHS was significantly reduced (determined by the ear swelling in vivo and the immune cell infiltrate in histology and flow cytometry). The hapten-specific Tc1-mediated T cell response was attenuated after hapten-specific restimulation in vitro (shown by T cell proliferation and Tc1 cytokine production). Comparing the two PAR2 transgenic mouse strains, the CHS reaction was significantly impaired in both of them, but G37I mutants had a less pronounced reduction than found in R38E mice. Interestingly, LysMCre/PAR2flox/flox mice (lacking PAR2 signaling in myeloid cells) exhibited a significantly impaired CHS reaction at 8 hours after the challenge, indicating a functional role of PAR2 signaling in myeloid cells in the early effector phase of CHS. Finally, our data indicate that TF and PAR2 play a key role in the pathogenesis of CHS and may be novel therapeutic targets in cutaneous inflammatory diseases.

P009 | Effects of water-in-oil ointments on skin barrier function and allergen penetration in an IL-31 treated 3D atopic dermatitis skin model

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Atopic dermatitis (AD) is a chronic relapsing, pruritic inflammation of the skin with dryness and disturbed skin barrier function and higher risk for allergic sensitization to environmental allergens, affecting 10-20% of children and 1-3% adults worldwide with increasing prevalence in highly industrialized countries.

IL-31, highly expressed in skin samples of AD patients, has been described as a mediator in AD. Recently, we established an IL-31 treated human 3D organotypic AD skin model and showed that IL-31 interferes with the differentiation of primary keratinocytes and inhibits the expression of terminal differentiation markers including filaggrin. In this study, we wanted to investigate the effects of two different water-in-oil ointments—one of which contains panthenol and ceramides—on the physical skin barrier structure and function in this AD model system.

We could reveal that physical skin barrier of the 3D skin model was recovered after daily topical treatment with the ceramide-containing ointment for 6 days. IL-31 treatment has been recently shown to downregulate filaggrin expression. However, topical co-application of both ointments prevented downregulation of filaggrin and disorganisation of other differentiation markers such as keratin 10 or β4-integrin as demonstrated by immunohistological analysis. Ki67 protein expression was also found to be upregulated after topical treatment with the ceramide containing ointment. This might correlate to the upregulation of amphiregulin expression detected by GeneChip Human Exon 2.0 ST microarray analysis and qRT-PCR. This protein has been shown to interact with the epidermal growth factor receptor to promote the growth of normal epithelial cells.

Furthermore, functional studies in IL-31 treated 3D skin models compared to untreated 3D skin models revealed an increased uptake of fluorescence labelled recombinant allergens of timothy grass (phl p1). Local application of the ointments over 6 days diminished this uptake and prevented increased transepidermal penetration of this allergen.

In conclusion, our data showed that the underlying inflammation and disturbed skin barrier induced by IL-31 allows increased transepidermal penetration of environmental allergens and, in collaboration with pruritus, probably facilitate inflammation and sensitization. Topical application especially of ceramide containing skin care ointments has been shown to reduce the IL-31 induced impairment of the physical skin barrier and skin barrier function in this AD in vitro model. This 3D AD model can be utilized in future to monitor ex vivo effects of various topical therapies on skin morphology, physiology, and gene expression.

P010 (OP04/03) | Epidermal barrier dysfunction in FlgHrnr-deficient mice promotes allergen sensitization and aggravates experimental asthma in a mouse model of MC903-induced atopic dermatitis

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Epidemiologic studies showed that atopic dermatitis (AD) often precedes other allergic disorders, eg, asthma, a process which is known as "atopic march." There is increasing evidence that AD is associated with defective skin barrier function. Loss-of-function mutations in the skin barrier proteins filaggrin (Flg) and hornerin (Hrnr) are a known risk factor for AD and for the further development of asthma. Filaggrin and hornerin are important for the terminal differentiation of the skin and the formation of cornified envelopes within the stratum corneum. We hypothesized that an acute AD-like phenotype in barrier-disrupted Flg/Hrnr−/− mice would facilitate sensitization to allergens and worsen clinical signs of asthma. To analyze the effect of skin inflammation on systemic sensitization, we sensitized wild-type (WT) and Flg/Hrnr−/− mice by ip, injections with ovalbumin (OVA) and subsequently exposed the mice to an OVA-aerosol challenge. Additionally, mice were treated topically with MC903 (calcipotriol; a low-calcemic analogue of vitamin D3) either during the sensitization or challenge phase. In the mouse, topical application of MC903 triggers an AD-like phenotype by inducing thymic stromal lymphopoietin (TSLP) in keratinocytes, which further aggravates allergic asthma.

Interestingly, Flg/Hrnr−/− mice treated with MC903 during systemic sensitization, but not during the provocation phase, showed significantly aggravated experimental allergic asthma. Flg/Hrnr-deficient mice had increased total cell counts in the bronchoalveolar lavage

(BAL) with a higher portion of inflammatory cells in the lung and an increased production of Th2-associated immunoglobulins, such as total IgE and OVA-specific IgG1.

The skin is a major allergen sensitization site, and skin barrier disruption promotes allergic sensitization by inducing systemic Th2 immunity, which in turn predisposes for allergic respiratory responses. Epicutaneous sensitization with OVA together with dibutylphthalate (DBP) results in an asthma-like phenotype after OVA-aerosol challenge. Of note, additional topical treatment with MC903 during sensitization appeared to increase asthmatic symptoms, and this effect was even more pronounced in Flg/Hrnr−/− mice.

In a model of MC903-induced AD, we showed that applying MC903 on impaired skin barrier during systemic as well as epicutaneous sensitization delivers signals that trigger the progression of asthma.

Thus, our study provides experimental evidence that skin barrier defects constitute an important risk factor linking AD to the atopic march.

P011 | From exposure to reaction: Panel study on the relationship between pollen exposure and the local and systemic expression of inflammatory parameters

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Background/aim of study: Pollen are one of the main causes of allergic airway diseases. Although it is clear that symptoms correlate with exposure to airborne pollen, to date, no information is available about relevant exposure thresholds. Furthermore, knowledge about changes in the allergen-specific immune response during the course of the pollen season is limited. Finally, pollen substances might affect the non-allergic population as well as non-allergenic compounds of pollen have been shown to have immune-modulatory and chemotactic effects.

Methods: In this study we assessed airborne pollen load in real-time (automated pollen counter) and by conventional method (Burkard traps). In an allergic rhinitis patient cohort and non-atopic control subjects we then monitored symptoms as well as the nasal and systemic immune response (activation of T cells and ILC2s, serum and nasal cytokines and chemokines, immunoglobulins) over the course of 1 year. Subgroups of patients and healthy control subjects were examined under allergen-free conditions at the research station Schneefernerhaus (Zugspitze) and were asked to keep a daily symptom diary. Immune monitoring was done in short intervals and included the determination of inflammatory parameters in nasal secretions, nasal curettages, serum and whole blood (ELISA, multiplex, transcriptomics). Nasal immune cell infiltrates will be characterized by flow cytometry. ILC2 numbers and allergen-specific T cell repertoire will be assessed in PBMCs. Analysis of cytokines and chemokines in serum and nasal secretions will be done by Multiplex or ELISA. Skin physiology (TEWL, skin pH) as well as cutaneous allergic immune response (skin prick tests) were assessed.

Results: Allergic patients benefited from staying in an allergen-free environment (Schneefernerhaus, Zugspitze) during peak pollen-season. Furthermore, out of pollen-season, allergic patients had lower symptom scores than non-allergic controls. In-season, the symptom scores of allergic patients are higher than the scores of non-allergics but they do not show a significant linear correlation with pollen count. In skin prick tests we observed a slight increase in histamine-induced wheal size in non-allergic subjects when comparing out-of-season to in-season results. TEWL and skin pH did not show any significant correlation to pollen counts. Serum IgE levels of allergic patients were transiently reduced after the stay on Schneefernerhaus and then increased again under high pollen exposure.

Outlook: In the future we hope to find correlations between pollen exposure and the allergic immune response, as well as to compare the results from the high altitude and urban conditions. Our aim is to understand more about mechanisms of the immune response during the pollen season. When is the first relevant contact with pollen exactly and what are the symptoms and allergic immune response? How to administer anti-allergic medication in the best possible way and when best to administer it? Final aim is to give valid advice to patients about relevant exposure thresholds.

* MG and SB contributed equally to this work.

P012 | IVDK data 2008-2013: Analysis of questionnaire and patch test results with regard to p-phenylenediamine (PPD) sensitization and cross-reactions

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Contact dermatitis is a widespread disease with an estimated lifetime prevalence of about 15% in Germany. P-Phenylenediamine (PPD) is a very frequent contact allergen and therefore is or was included in most national and international baseline series for patch testing. PPD allergy is predominantly caused by exposure to hair dresser products (especially hair dyes) and characterized by a manifold cross-reactivity to several para-compounds. Cross-sensitization may also results from exposure to textiles (eg, dispers colors) and leather (eg, shoes), fur, temporary henna tattoos, or even industrial rubber products. In order to identify risk factors for PPD sensitization (like exposure or occupation) the German Contact Dermatitis Research Group [Deutsche Kontaktallergie-Gruppe (DKG)] decided to include additional PPD-related questions into the standard questionnaire of the Information Network of Departments of Dermatology [Informationsverbund

Dermatologischer Kliniken (IVDK)] from 2008 to 2013. Within this period, 13 770 patients were patch tested with PPD (1% in petrolatum). 4293 of the PPD-tested patients were included in our analyses, because at least 90% of them (per department and quarterly period) have answered the PPD questions as well. This selection was performed to avoid bias in reaction frequencies, which can be introduced by different indications for patch testing or selected filling of questionnaires.

We compared the outcome of these questions between patients tested positively with PPD and those patients who reacted not positively. We clearly show that using hair color or being henna tattooed (at least once in a lifetime) represent significant risk factors for PPD sensitization. The allergic contact eczema of PPD-positives is predominantly located at the head (hairy or face) and is suspected to be particularly caused by hair cosmetics. Hence, especially hairdressers are at a high-risk for PPD allergy. However, private dyeing of the own hair seems not to pose a significant (additional) risk of sensitization to PPD in hairdressers.

Furthermore, by logistic regression analyses, we have estimated the influence of certain factors (sex, age, hair dyeing, henna tattoo, hairdresser profession) on cross-sensitization to para-compounds or coupled reactions to several other patch test substances than PPD. Hair dyeing predominantly elicits group allergy to para-compounds. The hairdresser profession is, beside the influence of PPD-related substances, characterized by sensitization to ammonium persulfate, which was not seen in dependency of the other factors estimated. Especially temporary henna tattoos are risk factors for multiple positive (cross-)reactions in young people. This comprises substances which are well known for cross-sensitization to PPD [eg, p-toluene diamine (PTD), p-aminophenol, dispers dyes], other substances without para-position of side chains (m-aminophenol) and substances which are not associated with hair dyes (hydroquinone) or the hairdresser occupation [N-isopropyl- N'-phenyl-p-phenylenediamine (IPPD)].

This study is not published so far and was not part of a submitted abstract elsewhere. By using epidemiological methods, we will illustrate the coherence between low/high doses of allergens on the skin of patients in the initiation phase and decreased/increased reactivity during elicitation phase and patch testing.

P013 | Age at disease onset has a major impact on clinical characteristics and course of mastocytosis

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The onset of mastocytosis occurs either in early childhood or adulthood. Recent studies demonstrated substantial differences between these two age groups. Childhood-onset patients usually exhibit cutaneous mastocytosis (CM) and often a transient course, whereas adult-onset patients usually have systemic mastocytosis (SM) and a chronic or progressive course. In the present study, we aimed to investigate the effect of age at disease onset on various disease parameters in a large cohort of patients with mastocytosis using a multicenter patient registry of the European Competence Network on Mastocytosis. Data of 1513 patients were entered into the registry by 20 specialized centers from Europe and the US. Our analysis revealed three age groups defined by specific disease characteristics. Patients diagnosed at age 0-16 years were characterized by CM and low tryptase levels. Patients diagnosed at age 17-59 years mainly showed non-advanced disease categories and a wide range of tryptase levels. In contrast, patients diagnosed at age ≥60 years often exhibited advanced SM categories, high tryptase levels, and elevated monocytes, eosinophils and alkaline phosphatase. Thus, we could demonstrate that there are also major differences within the adult population, in addition to the known differences between childhood- and adult-onset mastocytosis. Patients diagnosed at age ≥60 compared to 17-59 years develop more frequently advanced SM and should be monitored more closely.

P014 | Dopamine agonists block mast cell degranulation

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Mast cells (MC) are the key effector cells of allergic responses and responsible, at least in part, for the signs and symptoms of asthma, allergic rhinitis, atopic dermatitis and other allergic conditions. Following

their activation by cross-linking of the high affinity receptor for IgE, FcεRI, by IgE and antigen (allergen), MCs release multiple mediators, both preformed and newly synthesized. These cause vasodilation, sensory nerve activation and cellular influx by acting on endothelial cells, nerves, leukocytes and other cells.

To date, the symptomatic treatment of patients with MC-driven conditions relies on the use of antagonists to single MC mediators such as histamine (antihistamines). MC stabilizers are needed but not readily available for therapeutic use. By high-throughput screening we identified D1R agonists (D1RAs) as a possible new class of MC stabilizers with the potential to block IgE/antigen-induced degranulation and cytokine release. All 18 tested D1RAs led to reduction of calcium influx and at least 40% inhibition of MC degranulation as tested by beta-hexosaminidase and histamine release. In contrast, antagonists of D1R or compounds that target other dopamine receptors had no inhibitory effects. The D1RAs were active in the nM range and did not affect MC survival.

Our findings suggest that targeting of the dopamine pathway and D1Rs on MCs can be used to inhibit MC degranulation, which could enable the development of novel approaches for the treatment of MC-driven diseases.

P015 | IL-24 is a common and specific autoantigen of IgE in chronic spontaneous urticaria

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Chronic spontaneous urticaria (CSU) has been described to be linked to the presence of IgE autoantibodies (IgE-Aabs) to autoantigens (AAs). The prevalence, targets, and relevance of IgE-Aabs in CSU patients are largely unknown. Therefore, we screened CSU patients as well as two control populations for IgE-Aabs by high density protein array. We found 222 AAs to which CSU patients, but not control patients, have IgE-Aabs. 31 of these AAs were detected by IgE-Aabs in most (>70%) of CSU patients. Eight of these AA are accessible to IgE-Aabs, of which only one was a target of IgE in all patients: interleukin-24 (IL-24).

We developed an IgE-anti-IL-24-specific ELISA to measure serum levels in CSU patients (n=676) and healthy controls (n=456). In CSU patients and healthy individuals, the mean SD serum levels of IgE-anti-IL-24 were 0.410.37 IU/mL and 0.150.18 IU/mL, respectively. The cut-off was calculated to be 0.2 IU/mL. 470/676 CSU patients (70%) exhibited higher than normal IgE-anti-IL-24 serum levels as compared to 124/456 healthy controls (27%, $P<.0001$). IgE-anti-IL-24 showed good predictive properties for CSU, with a likelihood ratio of 2.4. We found that IgE anti-IL-24-sensitized human mast cells to degranulate in response to IL-24. Clinically, IgE-anti-IL-24 array signals showed a strong correlation ($r^2=0.84$, $P<.03$) with CSU activity determined by use of the urticaria activity score (UAS7).

Our findings show that CSU patients frequently exhibit IgE-Aabs against many AAs, and that IL-24 is a common and specific AA of IgE-Aabs in CSU.

P016 | The gasotransmitters nitric oxide and hydrogen sulphide block the degranulation of mast cells

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Nitric oxide (NO), carbon monoxide (CO), and hydrogen sulphide (H2S) belong to the family of short acting gasotransmitters. They quickly pass through cell membranes, are produced by endothelial cells of inflamed skin/mucosa, act on innate immune cells such as macrophages, and are reported to have clinical effects in cardiovascular disease. As of now, the effects of gasotransmitters on mast cells (MCs) have not been characterized in detail. Here, we investigated the responses of resting and IgE-activated human skin MCs to NO, CO, and H2S. CO, but not NO or H2S, induced the degranulation of human skin MCs and the subsequent release of proteases. Treatment with H2S and NO, but not with CO, reduced the spontaneous release of tryptase and beta-hexosaminidase in resting MCs by 20% and more than 50%, respectively. Similarly, H2S and NO, but not CO, reduced IgE-mediated MC degranulation and the release of tryptase and beta-hexosaminidase by up to 90%. In contrast, the IgE-triggered release of collagenase IV (MMP9) was not inhibited by H2S or NO. Taken together, we found that the gasotransmitter CO can cause MC degranulation, while NO and H2S inhibit the release of preformed mediators by resting and activated MCs. This might be relevant during the post-ischemic recovery of myocardial functional parameters and the repair of tissue injury.

P017 | Detection of proteins regulating mast cell adhesion and proliferation

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Little is known about the mechanisms of interaction of skin mast cells (MCs), key players in innate immune responses to pathogens and allergic reactions, with other cutaneous cell populations. Here, we used high-density protein microarrays to detect proteins to which MCs bind and assessed if they induced or inhibited MC proliferation. MC binding and proliferation was assessed by the use of a membrane-selective and a DNA-specific dye, respectively.

MCs adhered to multiple proteins, many of which had previously been reported to bind to MCs, eg, PECAM-1 and ICAM-1. Other prominent binding partners of MCs included stroma components as well as molecules

of the immune/hematopoietic system (eg, C-type lectins, interferons, IL-2, IL-21, IL-32, IL-37, CD52, stomatin, serum amyloids) and defensins (eg, dermcidin). Of note, many proteins were neuronal interaction partners (eg, neuritin, catenins, cortactin, neurotensin, galanin, filamins, plasmolipin, septin 1). The adhesion of MCs to several binding partners modulated MC proliferation. These included proteins that were known to induce MC proliferation, such as SDF1B, FGF8, IL-1F7, interferons, and IL-21, as well as novel inducers of MC proliferation (eg, Anxa11 and purinoceptors). Interestingly, we also identified several new binding partners of MCs that induce MC proliferation, for example kallikrein inhibitors such as cystatin E, pancipin or antiplasmin and ECM components like spondin 2, statherin or microfibrillar proteins. Taken together, we identified novel targets of MC binding, some of which modulate proliferation. These binding partners of MCs may be interesting in pathophysiology or as targets to manipulate the size of skin MC populations.

P018 | Integrin alpha E (CD103) is centrally involved in the regulation of dermal innate lymphoid cells type 2 in contact hypersensitivity reactions

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Dermal innate-like lymphoid cells (dILC2s) are increasingly recognized for their key role in development of allergies and eczematous skin diseases. Integrin alpha E (CD103), expressed especially by epithelial lymphocytes and dendritic cells but also by dILC2s, is thought to mediate adhesion and migration of T-cells in inflammatory skin reactions. We therefore examined the role of CD103+ dILC2 in murine contact hypersensitivity (CHS) models.

We found that CD103−/−/B6J mice display a significantly higher total dILC2 count compared to WT mice under physiological conditions. Interestingly, CHS triggered by topical treatment with DNFB, oxazolone, DNCB and FITC leads to similar inflammatory phenotypes in both mouse strains. While different CHS models lead to concordant up- or downregulation in total leukocyte count in WT and CD103−/− mice, absolute dILC2 count in WT mice were regulated identically compared to all leukocytes. Interestingly, total dILC2 numbers were left completely unchanged in CD103−/− mice in CHS. This observation seems to be specific in CHS, since irritant contact dermatitis induced by treatment with croton oil did not result in an increase of total dILC2 numbers in both mouse strains. The activation marker CD44 did not show significant differences on dILC2 in CHS of WT and CD103−/− mice. Our functional studies indicate that alterations in total dILC2 numbers were mediated by a combination of proliferation and migration involving integrin alpha E (CD103).

Our data suggest an important role of integrin alpha E (CD103) in dILC2 physiology specifically during development of CHS, but not during irritant contact dermatitis. Lack of CD103 seems to affect proliferation and migration of dILC2 in allergic skin reactions. A better understanding of the role of integrin alpha E (CD103) in dILC2 physiology might pave the way for therapeutic interventions in eczematous skin diseases.

P019 | Glycocalyx shedding in mastocytosis correlates with disease severity

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Mastocytosis is characterized by pathologic accumulation of mast cells in skin, bone marrow and other tissues. Cell membranes are covered by a glycocalyx consisting of various proteins. Under pathologic conditions, glycocalyx components can be released from the cell surface and accumulate in body fluids. In addition, shedding of the vascular endothelial glycocalyx may lead to increased serum levels of glycocalyx products. These shedding processes are incompletely understood, but mast cell-derived products may be involved. Therefore, we sought to investigate whether serum levels of glycocalyx components are altered in patients with mastocytosis and whether mast cell infiltrates express glycocalyx components.

We could demonstrate that serum levels of syndecan-1, heparan sulfate and hyaluronic acid are increased in patients with mastocytosis. In addition, we could show that skin mast cells are coated with glycocalyx components. We hypothesize that the mast cell glycocalyx is altered in mastocytosis. Alternatively, degranulation of perivascular mast cells may also affect the vascular endothelial glycocalyx.

Our findings suggest that serum levels of glycocalyx components might serve as novel diagnostic markers indicating disease progression in patients with mastocytosis. Moreover, stabilization of mast cells should be investigated in other diseases associated with alterations of the vascular endothelial glycocalyx.

P020 | Nod-like receptors and interleukin-1 cytokines in human basophils and eosinophils

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Human and murine neutrophils, monocytes and dendritic cells are known to express many pattern recognition receptors including extracellular Toll-like receptors (TLR) and intracellular Nod-like receptors (NLRs). Certain NLRs such as NLRP1, NLRP3, NLRC4 can activate inflammatory caspases via the formation of inflammasomes. These inflammatory caspases can then cleave and activate IL-1β.

While neutrophils and macrophages augment an inflammatory response directed against microbial or sterile danger signals, basophils and

eosinophils appear in allergic reactions such as anaphylaxis. Basophils can bind IgE on the cell surface and induce the secretion of mediators like histamine which lead to vasodilatation. Allergen specific IgE binds to the Fc receptors found on the surface of basophils and eosinophils. Upon binding of IgE to FcRε basophils degranulate. Although IgE-binding is the main cause of basophil degranulation cofactors such as bacteria or viruses are also known to induce mast cell and basophil degranulation.

Therefore we investigated the expression and function of several NLRs, inflammatory caspases and IL-1 cytokines in basophils and eosinophils and compared them to other myeloid cells by mRNA expression and protein secretion.

Freshly isolated basophils express most Nod-like receptors (NLRP1, NLRP3, NLRC4) that form inflammasomes stronger than non-basophils used as control cells. Upon LPS activation, basophils similar to other innate immune cells induce NLR transcription. NOD1 and NOD2 as intracellular receptors not implicated in caspase-1 activation are also present in basophils.

Interestingly, basophils do not express IL-1α and IL-1β and neither of the two cytokines can be induced by LPS stimulation, while IL-33 is only expressed by basophils and not by neutrophils.

Similar to basophils, eosinophils are not able to induce IL-1α and IL-1β synthesis upon TLR stimulation, while IL-33 is heavily induced by LPS treatment.

Although the mRNA level of IL-33 is high and IL-33 mRNA was translated into protein as the induction of IL-33 protein was observed in basophils in immunohistochemistry, we could never observe the secretion of IL-33 neither from basophils nor from eosinophils.

In conclusion, basophils and eosinophils as mediators of Th2 responses differ in the expression and activation pattern of IL-1 proteins, but not of NLRs which are involved in caspase-1 activation and consecutive IL-1 maturation.

Therefore the respective IL-1 cytokine (IL-1α/β vs. IL-33) in basophils or neutrophils might determine the respective T helper cell response in allergic diseases.

P021 (OP02/06) | Interaction between the microbiome and the transcriptome in lesional and nonlesional skin in atopic dermatitis patients

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Host-microbe interaction plays a critical role in the pathogenesis of atopic dermatitis (AD). It is unclear if changes in the microbiota affect the host immune status and/or skin barrier function, or vice versa, and how they together influence AD. We studied differences in the microbiome of lesional and neighboring nonlesional skin in AD patients and correlated them to changes in epidermal barrier and immune response-related gene expression in RNA sequencing transcriptome.

The microbiome in skin swab samples from AD patients (N=14, lesional site and adjacent nonlesional site) and seven healthy controls was sequenced using amplicon-based 16S analyses of hypervariable regions V1 to V3. The transcriptome was assessed by RNA sequencing from punch biopsies taken at the same lesional and nonlesional sites as the microbiome swabs.

The microbiota diversity in lesional skin was significantly low compared to nonlesional skin of AD patients. This was due to significantly higher frequency of the most abundant species in lesional AD samples, which is in 90% of the cases of the *Staphylococcus* genus. The frequency of several taxonomic units of *Staphylococcus aureus* is significantly higher in AD lesional samples, whereas in the AD nonlesional and healthy skin *Staphylococcus epidermidis* is the dominating species.

In contrast, transcriptome analysis showed a global difference between AD lesional and nonlesional skin samples. The majority of tight junction genes show a significant downregulation in AD lesional skin, whereas the cytokines IL-36G, IL-38 and IL-37 show upregulation.

Correlation studies between microbiome and transcriptome demonstrated significant positive association between the abundance of *S. aureus* with pro-inflammatory gene expression as well as with down regulation of tight junction expression. We further found a mixed pattern of negative and positive correlations between the abundance of *S. epidermidis* and the expression of interleukins and tight junction genes.

Our results show how gene expression differences in microbiome are correlated with inflammation status and skin barrier function. Furthermore, different Staphylococci species have opposite relationship to immune response and tight junction gene expression. Bioinformatics analysis allowed us to identify several groups of tight junction and immune related genes that differently correlated to the microbiome, potentially indicating distinct positive and negative influences on lesion development in AD.

P022 | Characterization of B cell responses in birch pollen-allergic patients treated by allergen-specific immunotherapy

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Background: It has been shown that allergen-specific immunotherapy (AIT) induces B cells secreting allergen-specific IgG antibodies and IL-10, pointing to a potential function of B lymphocytes in mediating allergen tolerance. In addition, allergen-specific IgG4 production seemed to be confined to a population of IL-10-producing B cells with immunoregulatory function. To further elucidate the role of B lymphocytes in tolerance induction, we investigated how the 3-year course of AIT influences the B cell activity in patients with birch pollen allergy.

Methods: Peripheral blood mononuclear cells (PBMCs) were isolated at several time points (baseline, month 1, 3, 6, 12, 18, 24, 30, 36) from birch pollen-allergic patients during a 3-year term of AIT. PBMCs were stimulated with IL-2 and R848 to quantify Bet v 1-specific antibody-producing cells (ASCs) by ELISpot analysis. Furthermore, separated B cells were incubated with CpG and anti-IgG to monitor IL-10 secretion. Finally, supernatants of PBMCs and B cell cultures were collected and analyzed by ELISA for production of allergen-specific IgG antibodies and IL-10, respectively.

Results: Enhanced numbers of Bet v 1-specific IgG-ASCs were noticed, which continued to rise during AIT, and were accompanied by an early increase of inducible IL-10-producing, peripheral blood B cells. Of note, cell frequencies evaluated by ELISPOT analysis coincided with both specific IgG and IL-10 levels measured by ELISA in the supernatants of the respective cell cultures.

Conclusion: Our findings suggest that long-term allergen tolerance induced by AIT is associated with an increase of IL-10-producing B cells capable to produce allergen-specific IgG antibodies. Thus, analysis of IgG and IL-10 responses on a single-cell level provides further insights into the potential immunoregulatory role of B cells in patients with immediate-type allergy.

P023 (OP03/06) | The Th2 chemokine CCL17 (TARC) production in human M2 macrophages is up-regulated by stimulating the histamine H2 receptor

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The histamine receptors present possible therapeutical target structures for the treatment of atopic dermatitis (AD). In allergic skin diseases such as AD, macrophages are attracted into tissue and exposed mainly to Th2 cytokines and also to histamine which is released in the skin during allergic reactions. CCL17 represents a key chemokine of M2 macrophages. CCL17 levels are increased in serum of patients with AD. Here, the expression of CCL17 in lesional skin of AD patients correlates with disease severity.

In this study, we investigated the role of histamine on human monocyte-derived M2 macrophages differentiated in the presence of M-CSF and activated with IL-4 or IL-13. H1R, H2R and H4R mRNA expressions were measured on fully differentiated and IL-4 activated monocyte derived M2 macrophages by quantitative PCR. We observed that the activation with IL-4 led to an up-regulation of the H2R and H4R at mRNA level in M2 macrophages.

The activation of M2 macrophages with IL-4 or IL-13 resulted in higher expression levels of the Th2 cell attracting chemokines CCL22 and CCL17 at mRNA- and protein level. Interestingly, the stimulation with histamine led to a significant further upregulation of CCL17 expression whereas the expression of CCL22 was not affected by histamine.

To show which histamine receptor is responsible for the up-regulation of CCL17, we stimulated the H1R, H2R and H4R on human IL-4 or IL-13 activated M2 macrophages with specific agonists. The stimulation of the H2R with the selective H2R agonist amthamine and 4-MH (H2R/H4R agonist) led to a significant time- and dose dependent up-regulation of the CCL17 expression at mRNA- and protein level in IL-4 or IL-13 activated M2 macrophages which could be blocked by pre-incubation with the specific H2R antagonist ranitidine. The H1R and H4R agonist did not show this effect.

In summary, we show a new function of the H2R by up-regulating the Th2 related chemokine CCL17 in human M2 macrophages which may lead to a pronounced attraction of CCR4 expressing Th2 cells into the side of inflammation and provide evidence for a role of histamine to support a Th2 dominated milieu. This may have an impact on the course of AD and for the treatment of the disease.

P024 | Human Th9 cells express functional histamine receptors

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Th9 cells are a distinct subset of CD4+ cells and develop in the presence of IL-4 and TGF-ß. They are involved in allergic inflammation by inducing mast cell growth and survival and facilitate the production of IL-13 and eotaxin. Moreover, it has been shown that IL-9 producing cells are increased in skin lesions of psoriasis and atopic dermatitis patients compared to healthy controls. As histamine is also upregulated in lesions of inflammatory skin diseases, we decided to investigate the role of histamine and its receptors on differentiation and regulation of Th9 cells.

Therefore naïve CD4+ T-cells, isolated from peripheral blood mononuclear cells (PBMCs) were cultured with IL-2, aCD3, aCD28, IL-4 and TGF-β for the differentiation into Th9 cells. Some cells were additionally incubated with histamine or specific histamine receptor (HR) agonists. After harvesting cells and supernatants, the expression of H1R, H2R and H4R as well as IL-9 production was measured. We detected an elevated expression of the H1R, H2R and H4R in differentiated Th9 cells compared to undifferentiated Th0 cells. Stimulation with histamine during differentiation led to an increase in IL-9 secretion compared to unstimulated controls. First experiments with specific HR ligands revealed a major role for the H4R.

Taken together our study demonstrates functional effects of histamine on Th9 cells.

P025 | Immune mechanisms induced by conventional allergen-specific immunotherapy and a newly developed hypoallergenic peptide-carrier fusion vaccine

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Introduction: Allergen-specific immunotherapy (AIT) is the only causal treatment for immediate-type allergies resulting in long-lasting immune alterations. However, since unwanted side effects can occur, it has been a matter of intense research to develop safer forms of AIT. One approach encompasses the development of a hypoallergenic peptide-carrier fusion protein. This vaccine is based on peptides from IgE-binding sites of the major grass pollen (GP) allergens fused to a Hepatitis B virus-derived non-allergenic carrier protein, PreS, which provides T cell help for the induction of allergen-specific blocking antibodies. Furthermore, the fusion protein shows a lack of IgE reactivity and a reduced potential to activate allergen-specific T cells. To evaluate the humoral and T cellular immune effects of this construct, GP-allergic patients undergoing a clinical phase IIb study of AIT with the peptide-based fusion vaccine were analyzed and compared to individuals receiving an AIT with conventional GP extract.

Methods: Patients were treated either with the peptide-based, hypoallergenic GP vaccine (Biomay, Vienna, Austria; n=5) or natural GP allergen extract (ALK-Abelló, Hrsholm, Denmark; n=8) over a 2-year observation period. GP-specific IgE, IgG and IgG4 antibody concentrations were quantified by ImmunoCAP. In addition, IL-5, IL-10- and IFN-γ-producing T cells were quantified after stimulation of peripheral blood mononuclear cells by ELISpot assay.

Results: Both cohorts of AIT-treated GP-allergic patients showed increasing concentrations of GP-specific IgG and IgG4 antibodies, which coincided with improved clinical symptoms, while IgE antibody concentrations remained unchanged. Interestingly, AIT resulted in enhanced allergen-specific IL-10- as well as IL-5- producing T cells in both groups during AIT, however these changes were only transient, returning to pre-treatment levels after cessation of therapy. In contrast, the frequency of allergen-specific IFN-γ-producing T cells did not change during the course of treatment.

Conclusion: The hypoallergenic peptide-based GP vaccine shows a potent capacity inducing allergen-specific IgG antibodies in GP-allergic patients, thus representing an interesting alternative treatment option for AIT. Of note, allergen-specific T cell responses seem not to differ in patients treated either with the hypoallergenic peptide or with natural GP extract, pointing to comparable immune alterations induced by these two forms of AIT.

CELLULAR BIOLOGY

P026 | Pharmacological targeting of glucose-6-phosphate dehydrogenase in human erythrocytes by Bay 11-7082, parthenolide and dimethyl fumarate

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In mature erythrocytes, glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) yield NADPH, a crucial cofactor of the enzyme glutathione reductase (GR) converting glutathione disulfide (GSSG) into its reduced state (GSH). GSH is essential for detoxification processes and survival of erythrocytes. We explored whether the anti-inflammatory compounds Bay 11-7082, parthenolide and dimethyl fumarate (DMF) were able to deplete a common target (GSH), and to impair the function of upstream enzymes of GSH recycling and replenishment. Reduced and oxidised glutathione was measured by HPLC and erythrocyte enzyme activities by spectrophotometric assays. 24 h treatment of erythrocytes (0.6% hematocrit) with Bay 11-7082, parthenolide or DMF lead to concentration-dependent programmed erythrocyte death (eryptosis), cell shrinkage, negligible hemolysis and complete depletion of both GSH and GSSG. Bay 11-7082 had the highest depletory effect on the intracellular GSH and GSSG concentrations. The complete depletion was achieved at 20 μM Bay 11-7082; a 2.5- fold resp. 7-times lower concentration as compared with parthenolide or DMF.

GSH depletion was due to strong inhibition of G6PDH activity. Both, 20 μM Bay 11-7082 or 50 μM parthenolide led to complete G6PDH inhibition whereas 140 μM DMF caused only 50% enzyme inhibition. Bay 11-7082 and DMF, but not parthenolide, were also able to inhibit GR activity. GR activity was completely inhibited by 20 μM Bay 11-7082 and only partially by 140 μM DMF.

In conclusion, the specific G6PDH inhibitory effect of these compounds may be exploited for the treatment of human diseases with high NADPH and GSH consumption rates. These diseases include malaria, trypanosomiasis, cardiovascular diseases, psoriasis, multiple sclerosis, cancer or obesity.

P027 | Biocompatibility and antimicrobial effects of a pulsed cold atmospheric plasma jet

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Introduction: Cold atmospheric plasma (CAP) consists of different components like free radicals and UV radiation. Due to their antimicrobial effect it becomes an interesting tool for wound treatment

and microbial-induced skin diseases. For medical application it is important to investigate the effects of CAPs on human skin to exclude cytotoxic reactions. This study examines the biocompatibility of CAP using a pulsed atmospheric plasma jet on human 3D-skin equivalents, which consist of epidermis and dermis. Furthermore, the antimicrobial activity against S. aureus, P. aeruginosa and C. albicans is examined.

Methods: For biocompatibility tests 3D-skin equivalents were treated with CAP using pulsed plasma MEF (Tigres, Marschacht). Plasma effects were investigated depending on the parameters process gas (air or nitrogen), input power and treatment times. 24 h after treatment 3D-skin equivalents were analyzed for cytotoxic effects, inflammatory reactions and morphological alterations. To determine the antimicrobial activity, microorganism were cultivated on MH2 agar plates and treated with CAPs. After 24-h incubation the zone of inhibition was evaluated.

Results: Low plasma doses or short treatment times exhibit good cell compatibility. Yet, skin models showed cellular damage as well as increasing release of inflammatory cytokines with higher dosage or longer treatment. The antimicrobial effect also depended on time and input power, increasing with both. Furthermore, it has been found that nitrogen as process gas is more effective than air.

Conclusion: Plasma application is a relatively new field of research in biomedicine. This study examined the effect of the pulsed plasma MEF technology on 3D-skin models and microorganism. Good biocompatibility could be confirmed in case of low plasma doses and treatment times. Nitrogen as working gas showed a distinct antimicrobial effect, even at low plasma doses. In summary, these results demonstrate that the generated plasma has the potential to become an effective tool for human skin decontamination as well as for wound management.

P028 | Free fatty acids boost the inflammatory response of immune cells within the skin

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Previous studies could show an exacerbation of skin inflammation, such as psoriasis, in relation with obesity. On the other hand, psoriatic patients have higher risk to become obese. Molecular mechanisms of these correlations are quite unknown. Thus, we would like to answer the question: how does obesity amplify skin inflammation?

We established an obesity mouse model, using high fat diet (HFD), with a psoriasis-like skin inflammation induced by topical application of IMQ. It could be shown that obese subjects develop a more severe skin inflammation as well on d3 (1xIMQ) as on d15 (5xIMQ), macroscopically seen and calculated as PASI-like score. Array analyzes of lesional skin revealed an increased expression in HFD-fed mice compared to chow counterparts as well of numerous pro-inflammatory cytokines and chemokines as receptors relevant for inflammatory signalling pathways.

Besides weight gain, impaired glucose metabolism, and an increase in body fat percentage, obesity is associated with an elevation of serum free fatty acids (FFAs). Thus, we investigated the role of palmitic acid (PA), as an example of saturated FFA (16:0), as a possible factor for the amplified immune response within obese subjects.

Especially, we focused on the effect of PA incubation of macrophages, which are key players in psoriatic pathogenesis. Surprisingly, PA stimulation alone did not affect the pro-inflammatory immune response of these immune cells. In contrast, pre-incubation with PA followed by LPS stimulation resulted in augmented secretion of TH1/TH17-instructive cytokines.

Our data suggest that increased levels of FFAs might be one predisposing factor for the enhanced pathogenesis of chronic inflammation, like psoriasis, in obesity. We could show a sensitization of psoriasis-relevant immune cells by FFA resulting in an exacerbated TH1/TH17 response.

P029 | Identification of the SPINK14 gene reveals RNA trans-splicing in normal keratinocytes

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The serine protease inhibitor Kazal-type (SPINK) gene family plays critical roles in skin homeostasis. Most of human SPINK genes are organized into a tightly linked cluster at chromosome 5q32 and encode proteins with one to several Kazal domains following the N-terminal signal peptide. We have previously identified SPINK6 and SPINK9. In this study, by using rapid amplification of cDNA ends followed by RT-PCR, we identified a novel member of the SPINK gene family, namely SPINK14, in the testis. Based on its full-length cDNA sequence, SPINK14 mRNA was generated by linear cis-splicing of five exons on chromosome 5q32, among which exons 2 and 3 encode an N-terminal signal peptide and exons 4 and 5 encode a typical Kazal domain. However, we did not detect this type of SPINK14 mRNA in the skin by RT-PCR with the same primers pairs.

To ask whether SPINK14 is expressed in skin, we performed 5′- and 3′-RACE PCR with poly(A) mRNA purified from normal human cultured keratinocytes and cloned its full-length cDNA sequence. To our surprise, SPINK14 displayed only an abnormal transcript in which a 117-nucleotide leader sequence was fused in frame with exons 4 and 5, leading to a coding frame for the identical Kazal domain but with lack of the signal peptide domain. By blasting against human genomes, the leader sequence could be mapped to a single locus on chromosome 7q11.23 where it comprises a remnant part of human endogenous retrovirus 1 (ERV1) sequence (67 bp) immediately followed by a complete Alu-Sq element. That suggests SPINK14 transcripts in keratinocytes (herein named as EA-SPINK14) could be derived from chromosomes 5 and 7.

To ask how EA-SPINK14 is produced, we first performed fluorescence in situ hybridization (FISH) on the cultured keratinocytes that had been confirmed to express EA-SPINK14 transcripts. By using two fosmid clones spanning 7q11.23 and 5q32, respectively, FISH did not detect any chromosomal rearrangement fusing the loci on 7q11.23 and 5q32. We then sequenced the 6-kb genomic region as indicated and did not find any DNA fragment associated with the 117-bp ERV1-Alu sequence. 3′-RACE analysis targeting the leader sequence identified ERV1-Alu RNA transcripts in the same cell line. ERV1-Alu RNA harbors a 5′ splicing donor site "GU" but has not a 3′ splicing acceptor site "AG," suggesting it could act as a spliced leader similar to that in lower eukaryotes. Interestingly, sequence analysis revealed an antisense Alu element located 95 base pairs prior to the start site of exon 4 of SPINK14, which might attract the ERV1-Alu RNA to the 3′ splice site. Taken together, the chimeric transcript EA-SPINK14 could be generated by the canonical trans-splicing reaction.

Next, we performed RT-PCR analysis using commercially available tissue RNAs. The expression of cis-spliced SPINK14 mRNA was restricted to the testis, while the chimeric EA-SPINK14 was present in many other tissues including skin, brain, colon, lung, kidney, bladder, thyroid, pancreas, small intestine, mammary gland and adrenal gland. Furthermore, in a commercially available cDNA library of human embryonic stem cells as well as healthy skin from six different individuals, we also detected only EA-SPINK14 but not SPINK14 transcripts.

In conclusion, our data provide evidence that trans-splicing naturally occurs in normal human cells. It might represent a genetic mechanism for a specific tissue to make a choice between cis- and trans-splicing of SPINK14. This finding may open up a new avenue in the investigation of functions and regulations of trans-splicing in human cells.

P030 | Necroptosis induces ADAM sheddase activity

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Necroptosis, is a caspase-independent form of programmed cell death executed by the receptor-interacting protein kinase 1 (RIP1), RIP3, and mixed lineage kinase domain-like protein (MLKL). Necroptosis-based cancer therapy has been proposed as a novel strategy for the killing of apoptosis-resistant cancer cells. Necroptosis has also been linked to many inflammatory skin diseases and may contribute to the pathogenesis of TEN (toxic epidermal necrolysis), a rare but potentially fatal drug hypersensitivity.

A disintegrin and metalloprotease (ADAM) 10 and ADAM17 are critically involved in regulating epithelial cell function and tissue homeostasis. They release epithelial cell adhesion molecules and ligands of the epidermal growth factor receptor (EGFR) including transforming growth factor (TGF)-alpha. Both proteases are known to be activated during apoptosis while their role in necroptosis is not clear. In this study, we set out to analyse whether necroptosis would induce ADAM10 or ADAM17 sheddase function.

First analyses were performed with the well characterized colonic epithelial cancer cell line HT29. Combined incubation with death ligand Killer-Trail, caspase inhibitor ZVAD and protein synthesis inhibitor cycloheximide induced necroptosis within few hours as evidenced by immunoblot analysis of pMLKL generation. We found that necroptosis led to significantly increased release of the ADAM substrate TGF-alpha. Analogous results were obtained with other cells and stimuli. TNF-alpha induced necroptosis led to increased IL-6 receptor shedding in the monocytic cell line U937. Next, we addressed the question how these effects could be explained. Activity assays with a soluble flourogenic ADAM substrate and ADAM inhibitors indicated that increased ADAM sheddase function was not due to changes in the bona fide enzymatic activity. Our recent findings indicate that exposure of the negatively charged phospholipid phosphatidylserine (PS) plays an important role in ADAM activation. Analysis of PS exposure revealed a prominent and early externalisation of this phospholipid during necroptosis.

In sum, our data indicate that necroptosis leads to enhanced ADAM sheddase activity and increased substrate release. It is tempting to speculate that this could contribute to the pathogenesis of inflammatory skin diseases and toxic epidermal necrolysis.

P031 | Osteopontin as a potential regulator of dermal ABCB5+ MSC maintenance

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We have characterized a novel population of multipotent mesenchymal stromal cells (MSCs) in young and old human skin, which hold substantial advantage over previously described MSC populations in their definition by a single marker, the P-glycoprotein ABCB5. Functional investigations revealed that ABCB5 is involved in cell cycle regulation. In situ, the dermal ABCB5+ MSCs co-expressed the established adult stem cell marker SSEA4 but not CD271, a nerve growth factor receptor whose expression is associated with neuro-ectodermal skin-derived precursors (SKPs) and malignant melanoma. Dermal ABCB5+ multipotent stromal cells (MSCs) showed a surface marker expression profile similar to conventional MSCs, immunomodulatory properties in macrophage activation distinct from dermal fibroblasts and are able to self-renew in vitro. In addition, a tripotent differentiation capacity into adipogenic, osteogenic and cartilage lineages was observed on a single cell-derived clonal level.

Several differences between ABCB5+ MSCs from young and old donors have been detected. For instance, dermal ABCB5+ stem cells isolated from humans above the age of 65 showed a gradual decrease in the percentages of cells in the bulk cultures expressing Sox2 and SSEA4 on

the protein level and deviant osteogenic and chondrogenic differentiation potential. Additionally, ABCB5+ MSCs isolated from old individuals presented with significantly increased base levels of DNA double-strand breaks. However, after exogenous induction of DNA double-strand breaks, both MSCs derived from young and old individuals repaired the damage with the same efficiency until the base levels of damage were reconstituted. Average telomere length of ABCB5+ MSCs derived from young and old donors were measured and found to be similar.

More detailed in situ characterization of the dermal MSC niche revealed a perivascular and interfollicular niche preference in young and old human and murine skin. In both organisms, we detected an age dependent significant decrease of the dermal MSC number, while in human the decrease was also concomitant with the change from a predominantly perivascular to a more interfollicular localisation. Interestingly, the decrease of ABCB5+ MSCs was correlated with a decrease of perivascular osteopontin, which is provided at least in human skin by perivascular NG2+ niche pericytes. In the complete absence of OPN in an OPN depleted mouse model we found even lower numbers of ABCB5+ MSCs compared to aged WT mice. This findings strengthened our hypothesis that OPN plays a pivotal role in dermal MSC biology. Further experiments will provide mechanistic insights into niche dependent paracrine regulation of stem cell biology by different protease cleaved osteopontin isoforms and their corresponding receptors on MSCs.

In a murine ABCB5 lineage tracing model the function of endogenous MSCs and osteopontin will be addressed using in vivo wound healing assays.

P032 | Evaluation of general risk and wound closure after cold plasma treatment in a dermal full-thickness mouse model

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Cold plasma as an alternative therapy option may be useful in the treatment of skin wounds. Previous studies have provided evidence that cold plasma supports the healing of wounds owing to its beneficial mixture of reactive species and modulation of inflammation in cells and tissues. To date cold plasma did display neither genotoxic nor mutagenic effects in human skin cells in vitro. However, in order to address this issue in vivo full-thickness wounds were created on ears of immunocompetent hairless mice (n=84). A significantly accelerated wound re epithelialization at day three to nine was demonstrated by transmitted light microscopy in comparison to untreated control animals after a daily cold plasma treatment over 14 days. After 1 year mice were investigated for tumor formation by non-invasive methods such as anatomical magnetic-resonance imaging (MRI) and positron emission tomography-computed tomography (PET/CT) with 18FFDG Tracer as well as by histological and immune histochemical analysis. Within the sensitivity and resolution limits of both modalities no apparent signs of tumor manifestation were found in any of the investigated structures and organs at the deliverable spatial resolution (MRI: 0.1 mm in-plane, PET/CT: 1.5 mm). Moreover, semi-quantitative PCR for different tumor markers (eg, α-fetoprotein, AFP; neuron specific enolase, NSE; carcinoembryonal antigen, CEA) confirmed no systemic long-term transformation in several tissues, organs and blood serum, no tumor and metastasis formation in the wound skin region of ears. Our results illustrate that cold plasma is a beneficial treatment option in wound therapies without an increased risk for transformational changes.

P033 (OP06/06) | Critical role of mast cells in epidermal barrier homeostasis

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The skin is a major interface between the organism and the external environment and a primary site of both sensing and inducing host defense responses. Acute and chronic epidermal barrier impairment are reflected in an increased transepidermal water loss (TEWL) and considered a pathogenic factor in an altered host defence and in inflammatory skin diseases (eg, atopic dermatitis). Increased histamine levels have been reported to impair epidermal barrier functions in a rodent model. In the present study we explored the role of mast cells (MCs) for basal epidermal barrier homeostasis and related functions as well as for the recovery following acute barrier disruption. Therefore we subjected two different genetically MC-deficient mice (KitWsh/W-sh and Mas-TRECK) to tape stripping.

We found no differences in basal parameters of TEWL or stratum corneum hydration between MC-deficient mice and WT mice. We assessed epidermal barrier recovery in response to tape stripping by TEWL measurements and histological analyses. Barrier recovery was significantly delayed in both MC-deficient mouse models compared to wild-type (WT) mice. A significantly delayed barrier repair was most prominent 24 hours after barrier disruption in KitW-sh/W-sh (−16.79 4.89 g/h/m^2) as well as Mas-TRECK (−9.56 6.51 g/h/m^2) mice compared to WT mice (P<.05). Adoptive transfer of bone marrow-derived cultured MCs as a reconstitution model into MC-deficient KitW-sh/Wsh normalised delayed barrier recovery to WT levels.

The MC-dependent delay in epidermal recovery was associated with epidermal hyperplasia measured by expression of Ki67 and increased epidermal thickness after 24 hours. Our data suggest that cutaneous MCs have a key role in enhancing epidermal barrier homeostasis and thereby might offer new possibilities for the prevention and/or treatment of pathological skin conditions.

P034 | Functional defects of dermal innate lymphoid cells type 2 (dILC2) in RAG1−/− mice determined by transcriptomic analyses

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Dermal Innate-like Lymphoid Cells Type 2 (dILC2s) are centrally involved in Th2-driven inflammatory diseases such as atopic dermatitis and allergy. RAG1−/− mice, lacking an indispensable factor in V(D)J-gene rearrangement, show higher relative and absolute dILC2 counts, which is why they are often used as functional models to study ILC2 physiology and pathology. 30-40% of ILC2 express RAG1 during their development, although they have no T or B cell receptor. Interestingly, it has been reported that RAG1 deficiency can lead to functional defects and altered gene transcription.

Although phenotypically similar (equal GATA3 and ICOS expression), contact hypersensitivity (CHS) models with DNFB lead to a dramatic decrease of dILC2s numbers in RAG1−/− mice, whereas these cells significantly increase in WT mice. Adoptive transfer of WT lymphocytes into RAG1−/− mice did not abolish the observed effect in DNFB CHS. We therefore searched for an intrinsic cause in dILC2. RAG1−/− dILC2s displayed impaired activation after in vitro stimulation with PMA/ionomycin. Moreover, we found a significantly higher baseline apoptosis rate in RAG1−/− dILC2. Lastly, RAG1−/− dILC2s showed an impaired proliferation rate.

We performed RNA multiarray analyses of dILC2 from RAG1−/− and WT mice. Surprisingly, it revealed significant expression alterations in over thousand genes, most of them related to immune response, cell cycle regulation and cytokine/cytokine receptor interactions. Among many others, Thy1 (CD90) and IL-7R (CD127) were differently expressed. IL-7R transmits a survival signal and induces expression of RAG1/RAG2. Thy1 has many physiological and pathological effects including apoptosis and activation regulation. FACS analysis confirmed the expression alteration on the protein level. Both of these protein changes could explain the massive physiological differences of dILC2s in RAG1−/− Mice.

In summary, our data suggest an important role of RAG1 and B/T cell interaction in dILC2 cell physiology beyond the well-known effects of RAG1 on T and B cell maturation. Numerous high-impact studies were performed on ILC2s of RAG1−/− mice and thus need to be carefully re-evaluated.

P035 (OP06/02) | Casein kinase II regulates the intracellular trafficking of the antigen uptake receptor DEC205 and is essential for effective antigen presentation by dendritic cells

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Guided by a 31 amino acid long intracellular domain, the dendritic cell (DC) receptor DEC205 takes antigens to MHC-II+ compartments in DC. The intracellular domain of DEC205 contains a protein kinase II (CKII) phosphorylation consensus sequence S-x-x-D. Here we asked whether phosphorylation by CKII is important for the routing of DEC205:antigen complexes to deeper endosomal compartments. We generated receptors containing the extracellular domain of human CD16 fused to the native intracellular DEC205 domain (CD16:DECwt) enabling us to use HuIgG as surrogate ligand. In some clones the CKII motive was rendered defective (CD16:DECδCK) by mutation. In the CD16:DECwt transfected model cell line DCEK a colocalization of HuIgG and CSKII became evident, suggesting involvement of CKII in intracellular antigen transport. This was further supported by results in DCEK cells transfected with CD16:DECδCK. Here reduced uptake of HuIgG as well as reduced MHC class- II mediated presentation of HuIgG was recorded as compared to CD16:DECwt transfected cells. As for the mechanism we found that CD16:DECδCK was trapped in the trans-Golgi compartments and did not recycle back to the cell surface during trafficking. To confirm these effects of CKII on DEC205 trafficking in vivo, we analysed mice deficient for CKII in DC (CD11cδCK). Here reduced expression of DEC205 in CD8+CD11c+ DC as compared to controls was evident. Moreover, when CD11cδCK mice were injected with OT-II T cells, followed by injection of CpG together with ovalbumin coupled to anti-DEC205 antibodies, we found drastically reduced in vivo proliferation of ovalbumin specific OT-II cells as compared to wild-type mice. Thus, these data establish for the first time a role of CKII in guiding endosomal trafficking of antigen uptake receptors in DC. Phosphorylation of the DEC205 receptor by CKII is a requisite for transporting DEC205 receptors back to the cell surface, which is mandatory for the effective uptake and presentation of antigens.

P036 | Cytoskeletal reorganization during NET formation

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The formation of neutrophil extracellular traps (NETs) is an immune defense mechanism of neutrophil granulocytes distinct from phagocytosis and ROS-mediated killing of pathogens. Through NETosis neutrophils are able to catch and kill various pathogens by expelling their chromatin and antimicrobial peptides. However, NETs also play an important role in autoimmune and inflammatory diseases. For this reason, unraveling the mechanism of NETosis has important clinical implications. NETosis is characterized by dramatic morphological changes in the cell's interior, including a massive reorganization of the cytoskeleton. Therefore, the detailed analysis of cytoskeletal

dynamics will contribute to the understanding of NET formation and provide insights into general principles of cellular morphogenesis.

To investigate the role of the cytoskeleton during NETosis, human neutrophils were analyzed in vitro after activation with phorbol 12-myristate 13-acetate (PMA). Cytoskeletal components such as alpha- and beta-tubulin and actin, proteins that link the cytoskeleton to the plasma membrane like ezrin, radixin and moesin as well as non-muscle myosin IIA were visualized by confocal microscopy in a time-resolved manner. Changes in the composition of the cytoskeleton were corroborated using Western blots. Moreover, the effect of different cytoskeletal inhibitors (blebbistatin, cytochalasin D, docetaxel, Y-27632) on NETosis was assessed at different time points.

Our results show that major components of the cytoskeleton such as microtubules and actin filaments, but also components of the actomyosin cortex are degraded during the course of NETosis. This degradation of cytoskeletal components is accompanied by cell rounding and significant softening of the neutrophil granulocytes. Inhibition of the microtubule apparatus by docetaxel does not have a significant impact on NETosis. However, actomyosin interactions appear to be necessary particularly during early phases of NETosis. As NETosis progresses, the functionality of the cytoskeleton is lost. It may be hypothesized that the loss of cytoskeletal integrity together with the alterations in the cell's shape and stiffness are mechanical requirements that allow for the final rupture of the cytoplasmic membrane and release of DNA.

P037 | Expression of the G protein-coupled receptors GPR109A (HCA2) and GPR43 is downregulated in psoriatic skin

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Recently it has been shown that butyrate, a bacterial product from fermentation of fiber in the colon, is involved in protection against colonic inflammation. The G protein coupled receptors GPR109A/HCA2 and GPR43 are the best known receptors for butyrate in the colon mucosa. The signaling of both promotes anti-inflammatory properties in colonic macrophages and dendritic cells and enables them to induce differentiation of IL-10 producing regulatory T cells (Treg). Skin autoimmune and chronic inflammatory diseases eg, psoriasis are driven by dysregulated Treg responses. Since stimulation of GPRs is necessary for homeostasis in the gut and their deficiency enhances susceptibility to colitis we asked whether a similar pattern can be found in psoriasis. To address this issue, biopsies from lesional and nonlesional skin of psoriasis patients and healthy controls were taken and analyzed for the expression of both receptors using immunofluorescence microscopy. The expression of GPR109A/HCA2 and GPR43 was significantly reduced in lesional skin compared to healthy control skin. The expression of both receptors in non-lesional skin was also decreased but to a lesser extent. Since GPRs are critically involved in the activation of peripheral Treg, a deficiency of these receptors might lead to an impaired function of Treg in psoriasis resulting in an enhanced inflammatory response in lesional skin. As ligation of GPRs promotes activation of gut Treg, we postulated that this process might also occur in the skin. For this purpose, skin biopsies from psoriasis and healthy skin were stimulated with sodium butyrate for 24 hours or left untreated. Immunofluorescence analysis revealed a significant upregulation of both GPRs upon butyrate treatment, suggesting that butyrate is able to activate local skin Treg via enhancing the expression of GPR109A/HCA2 and GPR43. The potential role of butyrate as an agonist of GPRs and inducer of Treg in inflammatory skin diseases provides new insights into the role of Treg in psoriasis and may allow developing new therapeutic strategies.

P038 | Water-filtered near-infrared a potential new treatment regime for keloids?

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Impaired wound healing, imbalanced dermal cell proliferation, imbalanced synthesis and degradation of extracellular matrix are associated with the development of hypertrophic scars. High recurrence rates, physical restriction as well as stigmatization are only some aspects influencing patients' life.

The panel of clinical applications of water-filtered near-infrared irradiation (wIRA) composed of near-infrared light (NIR) and a thermal component has increased in recent years. Due to the lack of conclusive discrimination between the thermal and the NIR component, we decided to investigate the impact of NIR on dermal cells exposed to different temperatures. Cell morphology as well as catabolic and anabolic processes of extracellular matrix proteins were monitored.

A keloid fibroblast cell line (KF111) was kept for 56 minutes at temperatures between 37°C and 46°C in a water-bath connected to a peristaltic pump. Keloid fibroblast cultures were in parallel exposed or not exposed to 360J/cm2 NIR generated by a wIRA irradiator. Cell morphology as well as the ability to re-attach after detachment were monitored every 2 h with an incubator microscope unit for 36 hours. Staining of cell nuclei with DAPI was used to monitor apoptotic and mitotic events. Collagen type I synthesis, cytokine secretion as well as selected signal transduction proteins of apoptotic pathways were monitored by ELISA.

Our results show that increased temperature induced morphological changes and decreased the re-attachment ability. Cell morphology changed from the usual rhomboid cell shape to a spherical shape. This morphological change was mostly reversed by co-stimulation with NIR. Cell nuclei staining showed that neither mitotic nor apoptotic structures were responsible for the rounded cell morphology. Detachment of the treated cultures directly after the treatment and re-seeding showed a temperature dependent decrease of cell re-attachment ability. The reattachment ability was significantly increased in cultures treated with the

combination of NIR and convective heat compared to cultures solely treated with heat. Likewise were collagen type I synthesis and TGF-β1 secretion regulated. Temperature dependent reduction could be partially restored by co-treatment with NIR. Neither convective heat nor the combination with NIR influenced the secretion of MMP-1.
The herein presented data suggest NIR in combination with heat as a promising therapy for hypertrophic scars.

P039 | Water-filtered near-infrared influences wound healing in vitro

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Wound healing is a complex process. Imbalance of cell proliferation, synthesis and degradation of extracellular matrix can cause eg, chronic wounds or the development of hypertrophic scars. The panel of clinical applications of water-filtered near-infrared irradiation (wIRA) composed of near-infrared light (NIR) and a thermal component has increased in recent years including wound treatment. Aim of our study was to characterize the thermal aspect as well as the influence of the light fraction of wIRA on dermal cells in context of in vitro induced wounds.
A wound situation was simulated by setting a scratch in confluent primary fibroblast cultures. These cell cultures were thereafter incubated for 56 minutes at temperatures between 37°C and 46°C in a waterbath connected to a peristaltic pump. Additionally the wound cultures were exposed or not exposed to 360J/cm2 NIR generated by a wIRA irradiator. Cell morphology and wound closure were monitored every 6 hours with an incubator microscope unit for 72 h.
Our results show temperature induced morphological changes. The initially rhomboid cell shape changed to a pronounced spherical cell shape. This morphological change was mostly abolished when co-stimulating with NIR. At physiological temperatures wound closure was enhanced by NIR in primary dermal cultures. Wound closure of cultures incubated at 46°C was completed after 72 h whereas co-treatment with NIR decreased the time needed for complete wound closure to 36 h.
The herein introduced in vitro treatment regime suggests a possible clinical application for chronic wound treatment. Further in vitro and in vivo studies will prove the effect.

P040 | Function of H2A deubiquitinase Mysm1/2A-DUB in skin pigmentation and melanoma growth

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The histone H2A deubiquitinase Mysm1/2A-DUB enzymatically removes ubiquitin from lysine residue K119 of the core histone H2A, an epigenetic mark that has been linked to transcriptional silencing. Based on our recent data revealing an interplay between Mysm1 and p53 in hematopoietic stem cells (HSCs), B cells and T cells we here investigated the role of Mysm1 in skin development and pigmentation and the involvement of this enzyme in tumorigenesis. In addition to the described hematological and mesenchymal stem cell phenotype of Mysm1−/− knockout mice we show here that Mysm1−/− knockout mice also present a white belly spot phenotype, implying a role for Mysm1 in melanocytic cell populations. Concomitant knockout of Mysm1 and tumor suppressor p53 provides a major rescue mechanism for the Mysm1−/− phenotypes. According to our findings, Mysm1 is transcribed and expressed in murine skin samples. Confirming the potential role of Mysm1 in the skin, histological analysis of skin sections from newborn Mysm1−/− knockout mice revealed a decrease in the epidermal layer thickness and an increase in dermal layer thickness. The altered morphology was accompanied by a reduced tyrosinase expression in the epidermal skin layer. The p53-mediated rescue in the hematological system of p53−/−Mysm1−/− double knockout mice was also effective in the skin of these mice. Skin morphology of p53−/−Mysm1−/− double knockout mice more closely resembled wild-type murine skin morphology as compared to Mysm1−/− knockout mice. Skin-derived Mysm1−/− knockout precursors displayed a reduced colony formation under melanocyte differentiation conditions in vitro compared to wild type. Using human melanoma cell lines we detected high expression of MYSM1 in A375 and SK-MEL-28 cells. Immunofluorescence staining of human skin sections indicated low expression of Mysm1 in healthy human skin and a high expression in human melanoma skin sections. In line with a potential role of Mysm1 in DNA damage repair, MYSM1 co-localized to sites of DNA damage upon etoposide treatment in human peripheral blood mononuclear cells (PBMCs) and human A375 melanoma cells as identified by the established DNA damage marker γH2AX. Lentiviral-transduced shRNA-mediated knockdown experiments in human A375 melanoma cells showed a reduced viability in MTT assays and an increase in apoptosis in Annexin-V/PI FACS experiments. MYSM1 knockdown also impaired the ability of A375 cells to grow anchorage-independently. Soft agar colony formation experiments showed a reduction in colony number and colony area in Mysm1 knockdown A375 cells as compared to control A375 cells. Initial studies from our group also indicated a regulation of MYSM1 by growth factors as well as regulation of proliferation by MYSM1. In FBS-starved A375 cells we could observe a striking reduction in MYSM1 protein levels and a restoration to normal growth culture levels upon re-stimulation with FBS after 6 hours of cell culture. Pax3 and c-Met have been implicated in melanoma cell survival and proliferation. In ChIP experiments in A375 melanoma cells we discovered an association of MYSM1 with the Pax3 consensus motif in the c-Met promoter suggesting a survival advantage for MYSM1 expressing melanoma cells as well as a possible role for Pax3 in during melanoma survival additionally to its role during melanocyte specification. In summary, this investigation reveals novel functions for the Histone H2A deubiquitinase Mysm1, previously mainly linked to murine hematopoiesis, in murine skin development and pigmentation

and in melanoma that may depend on interactions with the p53 pathway and c-Met thereby controlling apoptosis and cell survival.

P041 | Expression of fzd7 in epidermis is dysregulated with age and may have an impact on stem cell homeostasis

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Aging skin is characterized by a gradually increasing loss of the functional capacity of the epidermis, resulting in disturbed barrier function and impaired wound healing. We here addressed the question whether expression of the Wnt frizzled receptor fzd7 is dysregulated with age and whether this may impact on epidermal stem cell homeostasis and regeneration. In order to analyze fzd7 expression in differentiated and undifferentiated epidermal cells, we performed double immunostaining in skin obtained from young and aged C57BL/6J wild-type mice and assessed the colocalization of fzd7 with bulge and interfollicular stem cell markers eg, K15, CD34, LRIG, α6 integrin and differentiation markers eg, K10. Furthermore, the expression of fzd7 with age was evaluated by means of real-time qPCR and FACS analysis in young and aged murine epidermal basal cells (α6 integrin high cells) and bulge stem cells (α6 integrin high/CD34 high). To further assess whether fzd7 contributes to the undifferentiated state of epidermal cells, and whether this potential persists at high age, we examined the colony formation efficiency (CFU) of young and aged FACS sorted α6 integrin high/CD34 high/fzd7+ vs. α6 integrin high/CD34 low/fzd7+ vs. α6 integrin high/CD34-/fzd7- vs. α6 integrin high/CD34+/fzd7- epidermal cells in culture onto a feeder cell layer. Fzd7 was expressed in epidermal cells with consistent co-localization with stem cell markers eg, CD34, K15, LRIG and α6 integrin as well as with differentiation markers eg, K10 in young and aged epidermis. Of note, aged epidermal basal cells expressed significantly lower mRNA and protein levels of fzd7 and co-localization of fzd7 with CD34, K15, α6 integrin and K10 was also significantly reduced in these cells. Additionally, α6 integrin high/CD34 high/fzd7+ bulge stem cells were significantly reduced with age whereas á6 integrin high/CD34 low/fzd7+ epidermal cells showed a tendency to increase with age. Finally, expression of fzd7 regulated number and area of cell colonies grown in vitro. In aggregate, these data show that Fzd7 expression is disturbed in aged epidermis, and this may affect stem cell activity and the proliferation potential of epidermal cells. Based on these results fzd7 may qualify as a therapeutical target for the amelioration of wound repair.

P042 | Identification of essential and unique functions of mTOR signaling in skin development and homeostasis

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The epidermis serves as primary interface between the body and its environment and protects the organism from dehydration and external insult. As a stratified squamous epithelium the epidermis fulfils its function through a lifelong self-renewal process that is precisely coordinated by regenerative pathways, which in part recapitulate those that are also activated in epidermal morphogenesis. The exact mechanisms that orchestrate the fine-tuned balance between progenitor cell division in the epidermal basal layer and terminal differentiation of daughter cells within suprabasal layers remain to be determined. In this study we hypothesized a critical role of the mammalian target of rapamycin (mTOR) kinase in epidermal barrier formation and homeostasis. mTOR senses and integrates environmental cues from nutrients and growth factors, acting as important nexus for cellular signals to control growth and metabolism.

To dissect the role of mTOR pathway activation in epidermal development and homeostasis we specifically disrupted individual components of this pathway in mice by conditional gene targeting. TOR mediates its activities through the assembly of two structurally distinct multiprotein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), and we generated multiple mouse lines that specifically inactivated these complexes in the epidermis. We found that mTOR signaling is essential for skin morphogenesis as epidermal-specific Mtor mutants (mTOREKO) are viable but die shortly after birth due to lack of a protective epidermal barrier. As revealed by qRT-PCR analysis, Western blot analysis and immunohistochemistry phosphorylation of downstream targets of both mTORC1 (S6K, 4E-BP1) and mTORC2 (Akt-pS473, PKCα) were significantly attenuated in epidermal tissues of mTOREKO mutants. To determine whether mTOR function in epidermal development is primarily mediated by mTORC1 or mTORC2, in addition we generated mice with epidermal loss specifically for mTORC1 or mTORC2. Interestingly, epidermis-specific loss of Rptor (RapEKO), which encodes an essential component of mTORC1, confers the same skin phenotype as seen in mTOREKO mutants. In contrast, newborns with an epidermal deficiency of Rictor (RicEKO), an essential component of mTORC2, survive despite a hypoplastic epidermis. As revealed by BrdU pulse labeling, loss of epidermal mTORC1 activity attenuated basal cell proliferation, abrogated the epidermal stratification program and the formation of hair follicles. Furthermore, qRT-PCR and Western blot analysis of mTORC1- mutant epidermis revealed significantly attenuated expression of deltaNp63 isoforms. Consistently, deltaNp63 activated (Irf6, Gata3, Ikkα) or repressed (Runx2) target genes were attenuated or increased, respectively. In contrast, newborns of RicEKO mutants were characterized by a hypoplastic epidermis and increased transepidermal water loss. qRT-PCR analysis, Western blot analysis and immunohistochemistry of epidermis in RicEKO mutants revealed a significant delay in the initiation of the terminal differentiation program, whereas basal cell proliferation was comparable to controls.

Collectively, we provide genetic evidence for a fundamental role of mTOR signaling in the formation and maintenance of a protective epidermal barrier. We discovered distinct functions for mTORC1 and mTORC2 in skin barrier formation, which cannot compensate for each other. Our findings unravel important and novel mechanistic insights in epidermal development, maintenance and disease.

P043 | Alpha-ketoglutarate curbs differentiation and induces cell death in mesenchymal stromal precursors with mitochondrial dysfunction

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Increased concentrations of reactive oxygen species (ROS) originating from dysfunctional mitochondria contribute to diverse aging-related degenerative disorders. But so far little is known about the impact of distinct ROS on metabolism and fate of stromal precursor cells. We here demonstrate that an increase in superoxide anion radicals due to superoxide dismutase 2 (Sod2) deficiency in stromal precursor cells suppress osteogenic and adipogenic differentiation through fundamental changes in the global metabolite landscape. Our data identify impairment of the pyruvate and L-glutamine metabolism causing toxic accumulation of alpha-ketoglutarate in the Sod2 deficient stromal precursor cells as a major cause for their reduced lineage differentiation. Alpha-ketoglutarate accumulation led to enhanced nucleocytoplasmic vacuolation and chromatin condensation-mediated cell death in Sod2 deficient stromal precursor cells as a consequence of DNA damage, Hif-1α instability and reduced histone h3 (Lys27) acetylation. These findings hold promise for prevention and treatment of mitochondrial disorders commonly associated with aged individuals.

P044 | E-Cadherin is dispensable for epidermal localization of Langerhans cells

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Langerhans cells (LC) are potent antigen presenting cells localized in the epidermal layers of the skin. LC express high levels of the homophilic, calcium dependent adhesion molecule E-Cadherin (E-cad) which has been suggested to be responsible for adhesion to keratinocytes and thus LC localization to the epidermis. This is supported by three lines of evidence: a) formation of adherens junctions between E-cad expressing LC like dendritic cells and keratinocytes, b) down regulation of E-cad during activation, maturation and emigration of LC, and c) requirement of TGF-β for E-cad expression in dendritic cells (DC) and lack of epidermal LC in TGF-β null mutants. Since most of this evidence is indirect, we here address the question whether E-cad expression in LC is required for epidermal localization of LC in vivo. For this we used mice with a selective deficiency for E-Cad in CD11c + cells generated by Cre/LoxP-mediated recombination (E-cadfl/flCD11c+ Cre). In E-cadfl/flCD11c Cre+ mice E-cad expression was absent on epidermal and dermal dendritic cells (dDC) as demonstrated by flow cytometry (LC: CD11c+MHCII +CD207+EpCamhighCD103-; dDC: CD11c+MHCII+CD207+EpCamlowCD103+ and CD11c+MHCII+EpCamlowCD207-). Surprisingly, lack of E-cad in LC had only minor effects on epidermal localization of LC as demonstrated by only moderately reduced LC numbers in epidermal sheets and whole mount skin in Cre+ mice as compared to Cre- littermate controls. No differences were observed with regard to morphology, intraepidermal localization or activation status of LC under steady-state conditions. In addition, E-cadfl/flCD11c Cre+ mice displayed normal LC migration to regional lymph nodes as determined by in vivo FITC migration assays and normal ear swelling response in TNCB contact hypersensitivity. In contrast, in vitro significantly more LC could be isolated from epidermal sheets of E-cadfl/flCD11c Cre+ mice than that of Cre- controls, which may indicate that E-cad is involved but not essential for adhesion of LC to epidermal keratinocytes. In conclusion our findings reveal that despite the lack of E-cad LC are still largely present in the epidermis, suggesting that E-Cadherin is dispensable for localization of LC within the epidermal layers of the skin.

P045 | Epigenetic memory during cytokine-induced senescence in human cancer cells

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Cellular senescence has been linked to a number of physiological or pathological conditions such as aging, age-related diseases, embryogenesis, tissue homeostasis, and tumor development. In the case of tumor development, cellular senescence has been described as an important barrier that can be triggered by intrinsic or extrinsic pathways thereby curbing the unrestricted growth of the cancer cells. One of the best characterized exogenous triggers of senescence is a cytokine cocktail consisting of interferon (IFN)-gamma and tumor necrosis factor (TNF) which drives various murine and human cancer cells into permanent growth arrest in vitro. Until now, most research has treated cellular senescence as a binary on-off process, thus ignoring the temporal and hierarchical sequence of the process and the potential epigenetic memory effects that molecularly imprint the

induction of a former stress response. Here, we tested whether cellular senescence is subject to gene priming and epigenetic memory. For this, we treated cytokine-sensitive A204 rhabdomyosarcoma cells for 24-96 hours with IFN-gamma and TNF and then measured (i) cell proliferation by BrdU incorporation assay, (ii) SA-beta-galactosidase activity as a senescence marker and (iii) permanent growth arrest by determination of living cells after removal of the cytokines (growth assays). Treatment of A204 cells with the cytokine cocktail reduced cell proliferation by 70% after 72 hours, whereas the percentage of SA-beta-galactosidase-positive cells increased from 11% in controls to 42% in cytokine-treated cells. However, 72 hours of cytokine treatment were not sufficient to completely stop cycling of A204 cells, and the cells immediately restarted growing after removal of the cytokines, albeit at a slower rate. Thus, although 72 hours of treatment clearly induced senescence-associated cellular markers, the cells were not completely growth arrested but rather reached a senescence priming phase. Further experiments showed that induction of permanent growth arrest needed 96 hours continuous cytokine treatment or, alternatively, 48 hours continuous cytokine treatment after a 48 hours cytokine priming phase. Thus, cytokine-induced cellular senescence may indeed proceed via a molecularly defined time line with intermediate states of epigenetic memory. To unravel the cellular pathways and molecular networks leading to senescence priming and later on to decisive growth arrest of the cancer cells, we will analyse the kinetics of their transcriptome after continuous cytokine treatment, and then compare those kinetics with the respective kinetics of the priming, memory and senescence phases of the process.

Taken together, our data show that cytokine-induced senescence is a sequential process that may control malignant cancer growth. In contrast to lytic or apoptotic killing, senescence induction either needs long-term continuous treatment or intermitting short time exposure of the cytokines to completely stop cancer cell proliferation.

P046 | Staphylococcus epidermidis induces the host molecule A20 (TNFAIP3) to dampen induction of defense mediators in human keratinocytes

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Staphylococcus epidermidis is an abundant skin commensal capable of activating cutaneous defense responses. An example of innate defense responses elicited by S. epidermidis in keratinocytes is given by the induction of defense mediators such as cytokines and antimicrobial peptides. To permanently colonize human skin and to prevent unwanted induction of inflammation S. epidermidis needs to control its induction of defense mediators. Herein we report that S. epidermidis induces the expression of A20 (TNF-alpha-induced protein 3, TNFAIP3) in human primary keratinocytes thereby controlling the expression and release of IL-1-beta, IL-17C and human beta-defensin-2 (hBD-2). siRNA-mediated knockdown of A20 expression strongly enhances the induction of IL-1-beta, IL-17C and hBD-2 gene expression and protein release in keratinocytes stimulated with S. epidermidis. A20 regulates the gene induction of IL-1beta but has no influence on S. epidermidis-mediated proteolytic processing of IL-1beta. Mechanistically, A20 negatively controls the S. epidermidis-induced activation of the transcription factor NF-kappaB. Together, our data indicate that S. epidermidis exploits the host regulator protein A20 to attenuate cutaneous innate defense responses which may help S. epidermidis to persist as a commensal on human skin.

P047 | The impact of mitochondrial deficiency on dermal fibroblast function

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Fibroblasts are central regulators in skin homeostasis. They mediate the wound healing response and scar formation but also aging-associated pathologies. Yet, the underlying mechanisms by which the major cell type of the dermis meets these demands are poorly defined. In this study, we are aiming to investigate to which extend the function of dermal fibroblasts depends on intact mitochondria and which consequences a defective respiratory chain might have.

To answer these questions, we developed two different approaches. Wild-type dermal fibroblasts were isolated from newborn mice and treated with 10 mM sodium azide to block the respiratory chain. In addition, mice with fibroblast-specific accelerated accumulation of mtDNA deletions in fibroblasts were generated. For this purpose, we crossed mice that carry a targeted insertion of a dominant-negative mutant of the mitochondrial replicative helicase Twinkle with mice expressing a fibroblast-specific, tamoxifen-inducible Cre recombinase (Collagen Cre).

Our findings in vitro show that Sodium Azide treatment significantly reduces fibroblast proliferation. In addition, as revealed in a scratch-wound assay, fibroblast migration was significantly impaired by Sodium Azide treatment. Also fibroblast collagen interactions were disturbed as suggested from an altered collagen-lattice contraction assay when compared to controls. Together these findings indicate that key physiological functions of fibroblasts depend on proper mitochondria activity.

Currently, we are performing experiments to further analyze the effect of the accumulation of mtDNA deletions in dermal fibroblasts by activating the Twinkle helicase in vitro. Preliminary data suggest that the tamoxifen induced Collagen Cre positive fibroblasts deplete their mitochondrial DNA due to prior accumulation of mitochondrial DNA deletions, leading to a proliferation defect similar to the Sodium Azide effect.

It is well-established that during aging, tissues of mammals become mosaics of many normal and few cells with severe mitochondrial dysfunction. Future studies in gene modified Twinkle mice will help to

better understand mitochondrial function and dysfunction in dermal fibroblasts.

P048 | Antimycotic activity of Isatis tinctoria in an infected 3D model

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Introduction: Woad, Isatis tinctoria L., a blue indigo dye, has strong antimicrobial and anti-inflammatory properties most likely due to active compounds such as tryptanthrin. Hence, it is of great interest to investigate the antimycotic effect of woad-specific compounds especially for its application in formulations for adjuvant treatment of wounds and skin diseases. This study analyses the bioactivity and biocompatibility of tryptanthrin using an in-vivo-like 3D human skin equivalent cocultured with Trichophyton anamorph of Arthroderma benhamiae.

Methods: 3D human skin equivalents consisting of epidermis and dermis were infected with A. benhamiae DSM 6916 (1.0E+05 microconidia/mL) prior to tryptanthrin treatment for 1, 24, 48 and 72 hours. Analyses of cell viability, toxicity and inflammation were carried out. Expression rates of pro-inflammatory cytokines (IL-1α, IL-6, IL-8, IL-23α) and antimicrobial peptides (AMPs) such as h-BD2, h-BD-3, RNase7, psoriasin and TLR-2 were determined with qPCR. The skin models were further subjected to histological analyses.

Results: Infection of the 3D skin model caused slight toxic and inflammatory effects which were averted by application of tryptanthrin at a concentration of 250 μg/mL. Elevated gene expression of inflammatory cytokines after infection was decreased by tryptanthrin after 48 hours. However, it was still found to be increased after 72 hours at lower concentrations and only declined after application of 250 μg/mL tryptanthrin. AMPs were highly expressed under tryptanthrin alone and during infection.

Conclusions: It could be shown that tryptanthrin was able to decrease cytotoxic and inflammatory events in a 3D model during infection with A. benhamiae especially at higher concentrations. Further, tryptanthrin seemed to stimulate AMP expression even without pathogen contact. These results could suggest that tryptanthrin might have the potential to promote cellular defence and inflammatory response in human skin and could therefore be a natural antimycotic drug that supportively prevents skin from fungal infection.

P049 | Antimycotic activity of Isatis tinctoria in an infected 2D model

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Introduction: Woad, Isatis tinctoria L. (Brassicaceae), is known for its blue indigo dye and for its phytopharmacological properties. In addition, a strong preservative effect against fungal decay of wood has been observed. Active compounds, such as tryptanthrin, have been shown to exhibit strong anti-inflammatory properties as well as a broad antimicrobial spectrum. Hence, it is of great interest to investigate the antimycotic effect of woad specific compounds especially for its application in formulations for adjuvant treatment of wounds and skin diseases. The present study analyses the bioactivity and biocompatibility of tryptanthrin using an in vitro 2D model of human HaCaT keratinocytes co-cultured with Trichophyton anamorph of Arthroderma benhamiae.

Methods: HaCaT-keratinocytes were cultured for 48 hours and infected with A. benhamiae DSM 6916 (1.0E+05 microconidia/mL) before treatment with tryptanthrin for 24, 48 and 72 hours. Analyses of cell viability and toxicity as well as inflammation were carried out. Quantification of fungal growth was done by measuring calcofluor intensity and quantitative real-time PCR with genomic DNA.

Results: Infection of HaCaT with A. benhamiae caused antiproliferative effects after 24 hours and cytotoxic effects after 48 hours in the 2D-co-culture. Treatment with tryptanthrin led to an upkeep of cell viability. Furthermore, secretion of the pro-inflammatory cytokines IL-1α, IL-6, and IL-8 was distinctly reduced and fungal growth was nearly completely inhibited compared to the infected control.

Conclusions: This study showed that tryptanthrin is able to decrease cytotoxic and inflammatory events during infection with A. benhamiae in a 2D model. These results are crucial evidence that tryptanthrin with its cell protective and anti-inflammatory properties could be an effective compound that protects skin from fungal infection.

P050 | Topical rapamycin ameliorates imiquimod induced psoriasis-like skin inflammation in mice

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The mTOR (mechanistic target of rapamycin) inhibitor rapamycin has been long known for its immune suppressive properties but only showed limited therapeutic success when given systemically to psoriasis patients. Recent research has shown that the mTOR pathway is hyperactivated in lesional psoriatic skin, and in vitro data demonstrated that this aberrant mTOR signaling contributes to the disease by inducing hyperproliferation and disturbed maturation of keratinocytes. Thus, we suggest that topical mTOR inhibition could be a successful anti-psoriatic strategy. In order to test this concept in vivo, psoriasis-like disease was induced in dorsal skin of mice by daily topical imiquimod (IMQ) application in the afternoon for 4-5 days and 1% rapamycin or a vehicle control was applied in the morning within the same period. While dorsal skin fold thickness increased continuously

in the IMQ-treated animals over the course of the experiment, there was significantly reduced swelling in rapamycin treated animals. At the end of the experiment, the disease was clinically also less severe with less scaling and erythema. Reduced angiogenesis was found in the dermis and no splenomegaly could be observed.

Histological analysis revealed that rapamycin not only prevented the activation of mTOR signaling, that could be prominently seen in IMQ-treated animals, but almost normalized the epidermal phenotype concomitant with 50% reduction in the number of epidermal layers and microabscesses formation. The epidermal differentiation pattern was visualized by multispectral imaging of multiple overlapping markers such as keratins, involucrin and loricrin. While IMQ-induced lesions presented an abnormal expression and distribution of those markers, the normal epidermal differentiation pattern was almost restored to normal by rapamycin treatment. Moreover, the influx of IMQ activated innate immune cells in lymph nodes was reduced due to topical rapamycin, indicating its effectiveness as a topical agent. In summary, the data underline the role of mTOR signaling in the pathogenesis of psoriasis, and suggest to further investigate mTOR inhibition via topical application as a potentially successful strategy for anti-psoriatic treatment.

P051 | Inflammasome-independent IL-1beta processing by Staphylococcus epidermidis

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Staphylococcus epidermidis (S. epidermidis) is known as one of the innocuous commensals which abundantly colonizes human skin. Keratinocytes are able to sense bacteria to warrant a constantly regulated milieu of bacterial colonization. These controlling mechanisms can be mediated among others via the inflammasome, a multiprotein-complex that promotes the maturation of defense mediators such as IL-1beta through activated caspase-1. There is increasing evidence that sensing of S. epidermidis by keratinocytes is important to strengthen cutaneous innate defense through the induction of IL-1beta. Here we demonstrate that S. epidermidis is able to induce and process IL-1beta in an unconventional, inflammasome-independent manner.

Keratinocytes treated with living S. epidermidis or culture supernatants of S. epidermidis induced the secretion of the mature IL-1beta as well as the uncleaved pro-IL-1beta. To assess the participation of the inflammasome we down-regulated caspase-1 expression in keratinocytes using siRNA. This revealed only a marginal participation of caspase-1 in the maturation of IL-1beta. Thus we questioned if S. epidermidis itself is able to proteolytical process pro-IL-1beta to mature IL-1beta. Luciferase experiments with size fractionated supernatants of S. epidermidis revealed a pro-IL-1beta cleaving capability in the fraction >10 KDa. Western blot analyses confirmed pro-IL-1beta cleavage by >10 KDa supernatants of S. epidermidis. These observations led to the hypothesis that protease(s) produced by S. epidermidis may be responsible for the IL-1beta maturation. HPLC analyses of the S. epidermidis supernatants >10 KDa followed by mass spectrometry identified the S. epidermidis-derived serine protease Esp as the responsible protease which is able to process pro-IL-1beta. To verify the role of Esp in processing pro-IL-1beta we recombinantly generated Esp. Luciferase assays and western blot analyses using the recombinant Esp confirmed the capability of Esp to process pro-IL-1beta. In summary, we demonstrate for the first time that the S. epidermidis-derived serine protease Esp is able to generate mature IL-1beta in an inflammasome independent manner.

P052 | Loss of proteostasis as a pathomechanism in trichothiodystrophy (TTD)

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TTD is an autosomal recessive inherited disease characterised by a severe and varied phenotype. Symptoms usually range from delayed development, intellectual disability and cachexia to recurrent infections. Many patients do not survive infancy or early adulthood and about 50% of the cases have a photosensitive form of the disease. A hallmark of this disease is brittle hair that is sparse and easily broken. TTD is mainly caused by mutations in the xpb and xpd genes of the TFIIH complex, which is involved in DNA damage repair and transcription by RNA polymerases I and II. TTD also serves as a disease model for accelerated ageing and its study could help in understanding physiological ageing. In our study we show that cells from TTD patients suffer from a disturbed RNA polymerase I transcription leading to impaired ribosomal biogenesis and a compromised proteome. Reduced 18S rRNA levels and a reduced translational fidelity further reveal disturbances in the amount and quality of ribosomes. The loss of unfolding resistance of the proteome in the TTD cells leads to the onset of the unfolded protein response (UPR). The UPR in turn represses RNA polymerase I transcription resulting in a circulus vitiosus. In our study we show that the use of chemical chaperones can alleviate the ER stress and thus restore RNA polymerase I transcription in TTD cells. Our findings imply a possible treatment for this severe disease and might contribute to the understanding of the pathophysiology of the ageing body.

P053 (OP03/03) | A DNA repair-independent pathomechanism in Cockayne syndrome

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Cockayne syndrome (CS) is a genetic syndrome characterized by childhood onset of degenerative symptoms reminiscent of the aging body, such as loss of subcutaneous fat, alopecia, cataracts, neurological degeneration and cachexia. These symptoms are accompanied by developmental delay, resulting in a severe phenotype that can lead to childhood death. CS can be considered a model for accelerated aging and its exploration should foster our understanding of the physiological aging process.

CS can be caused by the recessive mutation of 5 genes (CSA, CSB, XPB, XPD or XPG) that are all involved in a branch of the Nucleotide-Excision Repair (NER) mechanism, thus explaining the elevated UV-sensitivity of the patients. However, total loss of NER is not always followed by premature aging, suggesting that alternative functions of the CS proteins have a crucial role in the disease.

One alternative function of the CS proteins is the transcription of ribosomal RNA by RNA polymerase I. Here, we show that a disturbed RNA polymerase I transcription in CSA and CSB-deficient cells is followed by a decreased translational accuracy of the ribosomes. This results in a high level of misfolded proteins and increased carbonylation of these proteins. As a result, ER stress and unfolded protein response are activated in CS cells and results in further repression of RNA polymerase I transcription, especially by activation of the protein kinase RNA-like ER kinase (PERK) pathway. CS cells also suffer from a high level of reactive oxygen species (ROS). The unfortunate combination of misfolded proteins and high ROS leads therefore to unresolved ER stress and to increased oxidative hypersensitivity in CS cells.

Our works shows that oxidative hypersensitivity of CS cells can be overcame by using chemical chaperones such as TUDCA (tauroursodeoxycholic acid). Moreover, TUDCA can decrease ER stress and restore the deficient RNA polymerase I transcription and protein synthesis of CS cells.

As these chaperones are approved by the FDA for the treatment of neurodegenerative diseases, our findings support a possible treatment for a devastating childhood disorder and may have an impact on our understanding of the molecular mechanisms underlying the aging process itself.

P054 | PPAR-alpha agonists suppress lymphangiogenesis

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Different pathologies, like lymphedema, cancer or chronic inflammatory diseases, are associated with abnormal lymphatic vessel formation. Therefore, influencing lymphangiogenesis is an interesting target. PPAR agonists are known to be effective anti-angiogenic and anti-tumorigenic agents. Up to now there is no evidence whether this property can be extended to an anti-lymphangiogenic action. To prove this assumption, we performed proliferation and functional assays with primary human dermal lymphendothelial cells (DLEC). We could demonstrate that only PPARα agonists suppresses DLEC proliferation, formation of capillary-like structures and migration. To examine whether these effects are conveyed by apoptotic mechanisms, we studied the amount of apoptotic nucleosomes and caspase 3/7 activity. There was significant apoptosis induced by PPARα agonists in DLEC. A cell cycle inhibition could be ruled out by FACS analysis. Since signaling via the vascular endothelial growth factor receptor (VEGFR1-3) pathway is critical for lymphangiogenic responses during chronic inflammation and tumor development, we explored whether PPARα agonists acted by diminishing VEGFR expression. Here, we could demonstrate a significant suppression of VEGFR1 protein expression. In contrast, neither VEGFR2 nor VEGFR3 expression was significantly affected by PPARα agonist treatment. Interestingly, the expression of VEGFR co-receptor neuropilin-2, known as an important regulator of lymphangiogenesis, was significantly suppressed. Hence, VEGFR-1 and neuropilin-2 expression may be critical molecular targets of PPARα agonists, which may be responsible for their anti-lymphangiogenic effects. Therefore, PPARα agonists are new anti-lymphangiogenic compounds and might be used in various illnesses associated with increased lymphangiogenesis.

P055 | Mitochondrial metabolic modulation: Potential influential factor in autoimmune blistering skin diseases

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Mitochondria being a central hub of cellular metabolism, their dysfunctions are causative for various pathologies. Mutations in the mitochondrial DNA (mtDNA) are known to cause such functional impairment in mitochondria. Indeed, we previously reported an association between the mitochondrial ATP8 synthase gene (MT-ATP8) and bullous pemphigoid in Germans, and additionally demonstrated that a conplastic mouse strain carrying a single mutation in the mt-Atp8 gene (B6-mt FVB) exhibited significantly less disease severity in experimental epidermolysis bullosa acquisita (EBA).

To study the functional consequence of the mt-Atp8 mutation, we investigated cellular metabolism in B6-mt FVB mice, since it has shown that the alteration of metabolism and metabolites is a critical determinant in cellular functions.

Short chain fatty acids (SCFA) are known to affect cellular functions in various cell types. Therefore, we evaluated the levels of a panel of metabolites in different tissues in B6-mt FVB and B6 (wild type). The levels of propionate (C3) were significantly higher whereas those of acetate (C2) and butyrate (C4) were significantly lower in B6-mt FVB compared with B6 in liver, skin and lymph nodes ($P<.05$, in each metabolite and respective organ, t-test). Additionally, we performed cellular flux analysis to evaluate cellular metabolism in lymphocytes and skin fibroblasts in both strains. Mitochondrial respiration in the

mt-Atp8 mutant strain is impaired as levels of basal oxygen consumption, OXPHOS-dependent ATP production, and spare capacity were significantly lower than those in wild-type cells (decrease by approximately 20% in B6-mtFVB compared to wild-type, respectively, $P<.05$), suggesting limited capacity of cellular activities (ie, cell proliferation) in mt-Atp8 mutant cells.

These findings indicate that the skewed cellular metabolism caused by the mt-Atp8 mutation may result in beneficial effects in experimental EBA. As such modulation of mitochondrial cellular metabolism could be therapeutic or even prophylactic option in AIBD.

P056 | Arsenic trioxide decreases lymphangiogenesis by inducing apoptosis and inhibition of important lymphatic endothelial cell receptors

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Background: 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, influencing lymphangiogenesis is an interesting target in cancer therapy. Signaling via the vascular endothelial growth factor receptors-2/-3 (VEGFR-2/3), Lyve-1 and Tie-2 pathways are critical for lymphangiogenic responses. Arsenic trioxide (As2O3), which is used as an effective treatment against relapsed acute promyelocytic leukemia, is characterized by low cytotoxicity. As As2O3 promotes anti-angiogenic effects on endothelial cells, we hypothesized that As2O3 may have impact on lymphangiogenesis. Therefore, we explored whether the known antitumorigenic properties of As2O3 might be additionally mediated in part by antilymphangiogenic effects through the reduction in VEGFR-2/3, Lyve-1 and Tie-2 expressions in primary human lymphatic endothelial cells.

Methods: Human lymphatic endothelial cells (LEC) were cultured in vitro and treated with or without As2O3. Effects of As2O3 on proliferation, apoptosis and expression of the important endothelial receptors VEGFR-2/3, Lyve-1 and Tie-2 were analyzed mainly by BrdU-Assay, cell death assay, caspase-3/7 activity assay, cytochrome c-ELISA and immunoblotting. In vitro angiogenesis was investigated using the matrigel tube formation assay.

Results: As2O3 inhibited cell proliferation in a concentration-dependent manner. In our study we found that As2O3 induced apoptosis by activating Caspase-3/-7 and cytochrome c release in LEC. In addition, we could demonstrate an inhibition of the formation of lymphatic capillary like structures by As2O3 treatment. Furthermore, we demonstrated that As2O3 significantly inhibited VEGFR-3, Tie-2 and Lyve-1 protein expression whereas VEGFR-2 expression was unaffected after treatment with As2O3.

Conclusion: In conclusion, our results provide for the first time clear evidence, that As2O3 has distinct anti-lymphangiogenic effects mainly by inhibition of the endothelial VEGFR-3, Tie-2 and Lyve-1 as well as apoptosis.

P057 | Identification of keratin K23 as a component of the cytoskeleton in cornifying epidermal keratinocytes

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Terminal differentiation of keratinocytes involves major changes of the keratin cytoskeleton. Here, we investigated the expression of the type I keratin K23 which had previously been detected in simple epithelia under stress conditions. Differentiation of normal human epidermal keratinocytes in vitro led to a more than 200-fold upregulation of K23 mRNA levels, as determined by quantitative RT-PCR. Using a newly raised anti-K23 antibody, K23 protein was detected, by Western blot analysis, in the cytoskeletal protein fraction of confluent keratinocyte cultures and, by immunohistochemistry, in the granular layer of human epidermis. Thus, the expression pattern of K23 paralleled that of the type II keratin K2. Comparative genomics showed that K2 is specific for mammals whereas K23 orthologs are present not only in mammals but also in other vertebrates, indicating that K23 is an evolutionarily ancient keratin. Our results define K23 as a marker of keratinocyte terminal differentiation and suggest that this keratin contributes to the cytoskeletal maturation during the formation of the epidermal barrier to the environment.

P058 | Tyrosinase-Cre-mediated deletion of the autophagy gene Atg7 leads to aberrant accumulation of p62/sequestosome 1 in melanocytes of the skin and in neurons of the brain

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Many mouse models for the investigation of autophagy are based on the cell type-specific deletion of essential autophagy genes using the Cre/loxP system. We have previously generated tyrosinase (Tyr)-Cre Atg7f/f mice in which the tyrosinase promoter directs the expression of the Cre recombinase to pigment cells. Cre-mediated deletion of the floxed Atg7 gene suppresses autophagy and dysregulates the antioxidant response of epidermal melanocytes in these mice. As the tyrosinase promoter was also reported to be active during the development other neural crest and neuroepithelial-derived cell

types, we investigated whether tyrosinase-Cre Atg7f/f mice have a brain phenotype. Indeed, the brain of Tyr-Cre Atg7f/f mice, but not that of control mice, showed accumulation of the autophagy adaptor and substrate p62/sequestosome 1. p62-positive protein aggregates were detected in subsets of neurons in the cortex, basal ganglia, nucleus subthalamicus, substantia nigra, and thalamus. The number and size of p62-positive aggregates increased with the age of Tyr-Cre Atg7f/f mice. In contrast to mouse models lacking autophagy in all neurons, the alterations of Tyr-Cre Atg7f/f neurons were not associated with overt abnormalities in health and behavior of mice up to an age of 2 years. These results suggest that the suppression of Atg7-dependent autophagy in parts of the brain makes Tyr-Cre Atg7f/f mice a new model for the study of autophagy-related processes during aging of the brain.

P059 | Inflammation-dependent activation of mTOR signaling induces the antimicrobial peptide koebnerisin (S100A15) to control epidermal maturation in psoriasis

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The mTOR (mechanistic target of rapamycin) pathway is a central regulator of cell growth and differentiation. In psoriasis, the mTOR kinase is activated throughout the epidermis, particularly in the basal proliferating layers. To date, disease-intrinsic factors, which regulate epidermal mTOR activity, are yet to be identified. Koebnerisin (S100A15) is an innate anti-microbial and immune-modulatory peptide strongly upregulated in the psoriatic epidermis.

Data revealed that the epidermal distribution of koebnerisin resembles the activation pattern of mTOR kinase in psoriasis. Thus, we hypothesized a functional link and could show an inflammation-dependent induction of koebnerisin via IL-1β, TNF-α and IL-17A, which strongly depended on mTOR signaling in keratinocytes. In turn, the enhanced secretion of koebnerisin led to activation of mTOR signaling via the PI3-K/Akt cascade. Functionally, koebnerisin supported keratinocyte proliferation, which underlines the role of mTOR signaling in regulating epidermal proliferation. In addition, the koebnerisin induced mTOR activation interfered with the protein levels of differentiation markers in keratinocytes, such as involucrin and filaggrin. Interestingly, the transcriptional levels of differentiation markers remained unchanged suggesting a post-transcriptional mechanism of regulation via TOR during epidermal maturation.

Using koebnerisin (S100A15) as an example, we provide mechanistic evidence that anti-microbial peptides induced by inflammatory cytokines contribute to the disturbed epidermal maturation in chronic skin inflammation. These findings further emphasize the mTOR network as a therapeutic target in chronic inflammatory diseases in the skin and beyond.

CHEMOKINES/CYTOKINES

P060 (OP05/06) | Skin and neutrophil derived proteases regulate the activity of IL-36 family members

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The pro-inflammatory cytokines IL-36α, IL-36-β, IL-36-γ are IL-1 family proteins highly expressed by epithelial cells. IL-36γ is of particular clinical relevance as its aberrant expression is emerging as a specific mediator of psoriatic inflammation and mutations in the IL-36 receptor antagonist which inhibit IL-36-mediated signalling presents with pustular psoriasis phenotypes. As with IL-1β, the activity of IL-36 cytokines is known to be regulated through the proteolytic cleavage of inactive pro-forms into processed bioactive proteins. However unlike the well characterised IL-1β activation mechanism, caspases are not involved. Our aim was therefore to identify proteases capable of IL-36 activation and to explore the importance of cytokine activation in psoriasis.

Using a keratinocyte-based activity assay in conjunction with small-molecule inhibitors and siRNA gene silencing, cathepsin S was identified as the major IL-36γ- activating protease expressed by epithelial cells. Interestingly, both cathepsin S activity and IL-36γ expression were strongly upregulated in samples extracted from psoriasis patients, and IL-36γ Ser18, the major product of cathepsin S processing was shown to induce psoriasiform changes in a human skin-equivalent model.

In addition to the involvement of skin-derived cathepsin S, we also found that neutrophil elastase rapidly activates the IL-36 receptor antagonist, whilst prolonged exposure leads to the inactivation of all IL-36 members. Together these data show that keratinocyte derived cathepsin S plays a key role in the activation of IL-36γ and psoriatic inflammation, and that neutrophil-derived proteases may mainly act to regulate the network of IL-36 activity through the activation of the receptor antagonist and the degradation of IL-36 agonists.

P061 | Analysis of soluble wound material obtained from acute and chronic wounds

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Non-healing wounds are a major thread for the well-being of patients. These wounds are characterized by a low-grade, steady-state inflammation, and do not transit to the proliferation phase. Wound healing phases are controlled by a number of factors, including wound

resident skin cells, immune cells, cytokines and chemokines as well as underlying diseases such as diabetes or bacterial infection. To better understand the contribution of soluble factors in proper and defective healing, wound exudate material was collected from about a dozens of patients each having surgical acute wounds and diabetic chronic wounds. The concentration of 37 different cytokines and chemokines were assessed in both types of samples and compared to each other. Also, cellular content was investigated by flow cytometry. At the same time, concentration and origin of small particles such as microparticles (<1 μm) were assessed utilizing a flow cytometric approach. Both types of wounds were markedly different regarding the cytokine and chemokine pattern as well as cellular and small particle content. These results may help to better understand the difference in quality of inflammation present in pathological wound healing, possibly allowing for therapeutic intervention in the future.

P062 (OP04/02) | IL-17A and IL-36γ act in a synergistic manner and induce differentiation defects in 3D skin models

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IL-17A has been identified as a key cytokine in the pathogenesis of psoriasis. Therefore we investigated the effects of IL-17A on downstream molecules and on the formation and the functionality of the skin barrier in human organotypic 3D skin equivalents.

We treated two different types of 3D models of human epidermal keratinocytes (NHEKs) with IL-17A and compared these to untreated models. For that we used psoriasis models developed with NHEKs and dermal fibroblasts from psoriatic lesions of patients and control models containing cells from healthy donors. Stimulation with IL-17A revealed changes in skin morphology in both models including parakeratosis. Microarray analysis of these models revealed down-regulation of genes important for epidermal differentiation and skin barrier formation and an increased expression of different antimicrobial peptides (AMPs), including human beta defensins (hBDs) and members of the S100 calcium binding family upon IL-17A stimulation. Furthermore we found an up-regulation of different chemokines and cytokines like IL-1β, CCL20 and CXCL17 as well as of all members of the IL-36 cytokine family in IL-17A treated 3D models. Thus it is tempting to speculate that the IL-17A effects were at least in part mediated by the induction of IL-36 cytokines in keratinocytes. Indeed their application was sufficient although to a lower extent compared to IL-17A to induce the expression of genes encoding different AMPs, including S100A7A and hBD-2 or chemokines like CCL20. We could show that IL-36 cytokines are produced and secreted upon IL-17A stimulation in NHEKs and 3D models and that these proteins are unprocessed and therefore almost inactive. We were able to activate the released IL-36γ with the addition of activated neutrophil supernatants or recombinant neutrophil elastase. In both approaches we could detect an increased expression of genes encoding AMPs and IL-36 cytokines. Furthermore the activated IL-36γ in combination with IL-17A showed synergistic effects on gene expression in NHEKs. In addition IL-36γ can also activate the expression of IL-17C and seems to be part of an amplifying mechanism. Therefore we hypothesize that IL-36 cytokines are produced upon IL-17A stimulation and activated through neutrophil proteases to enhance the psoriatic phenotype.

In conclusion we were able to establish 3D organotypic skin equivalents with NHEKs and fibroblasts of psoriasis patients. In these as well as in control models IL-17A disturbed differentiation. The analysis of the downstream consequences showed that IL-36 cytokines are produced but need to be activated via neutrophil proteases to enhance the IL-17A effects.

P063 | Characterization and modulation of CC-chemokine receptor 6 (CCR6) mediated immunosurveillance in malignant melanoma

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Chemokine ligand 20 (CCL20) and the antimicrobial peptide ß-defensin expressed in the epidermis are a potent impetus for the recruitment of subsets of dendritic cells (DC), B-cells and memory T cells expressing chemokine receptor 6 (CCR6), the sole cognate receptor. In addition to its constitutive expression in the epidermis, CCL20 and a corresponding CCR6-expressing immune cell infiltrate have been detected in several malignancies, including melanoma. Yet, the functional contribution of the CCR6/CCL20 axis for the immune control of melanoma remains controversial. The characterization of CCR6-guided immune cell subsets and their functional contribution for the immune control of melanoma comprises the focus of this project.

We evaluated the homeostatic and inducible secretion of CCL20 by different murine and human melanoma cutaneous cell lines by enzyme-linked immuno-absorbent assay (ELISA). Both, murine (B16, Ret) and human (A375, C32) melanoma cell lines are capable of secreting CCL20 and show significant differences in the magnitude of inducible CCL20 expression upon stimulation with pro-inflammatory cytokines (ie, TNF-α, IL-1α, IL-1β and TGF-β) in vitro. In order to determine the functional relevance of CCR6 on local tumor growth, metastasis and the tumor microenvironment, B16 melanoma cells retrovirally transduced with a vector that constantly overexpresses CCL20 (B16-CCL20) were injected subcutaneously into the flank of wild-type C57BL/6 (WT) and congenic CCR6-knockout (CCR6KO) mice. While animals in both groups developed local tumors, we observed a significantly reduced tumor growth in CCR6KO mice. By contrast, WT and CCR6KO control groups (injected with a B16 line that does not express CCL20) did not display differences in tumor growth rate. While the precise mechanisms require further investigation, our

results suggest that CCL20/CCR6 interactions in the microenvironment of cutaneous melanoma may be an essential factor for local tumor growth.

Current experimental approaches focus on the characterization of tumor infiltrating immune cells in cutaneous melanoma of both WT and CCR6KO mice by means of fluorescence-activated cell sorting (FACS) and the relevance of a functional CCR6/CCL20 axis for local and distant metastases.

P064 | Elevated levels of IL-17A in the blood and skin of patients with bullous pemphigoid

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Bullous pemphigoid (BP), the most frequent autoantibody-mediated blistering skin disease, mainly affects elderly patients. While current treatments are based on the long-term use of superpotent topical or systemic corticosteroids more specific therapeutic strategies with less adverse reactions are urgently needed for this group of patients. Immunopathologically, BP is characterized by autoantibodies against two structural proteins of the dermal-epidermal junction, collagen type XVII (BP180) and BP230. Binding of anti-BP180 autoantibodies causes an inflammatory cascade leading to local expression of pro-inflammatory mediators and proteases that finally cause subepidermal blistering. IL-17A is a pro-inflammatory cytokine that eg, induces expression of chemokines in keratinocytes and recruits and activates neutrophils. So far, only limited information is available about IL-17 and IL-17-related cytokines in BP.

We observed significantly elevated levels of IL-17A (P=.02), IL-21 (P=.01), TNF-á (P=.04), and IL-6 (P=.02) in the serum of patients with BP (n=15) compared to age- and sex-matched patients with non-inflammatory dermatoses (n=10). When analyzing IL-17A+ immune cells in the peripheral blood we identified CD4+ lymphocytes (P=.01), but not CD8+cells (P=.9), monocytes (P=.1), and neutrophils (P=.7) to be increased in BP patients (n=15) compared to the above mentioned control group (n=10). By immunohistochemistry of perilesional skin biopsies of BP patients (n=7) CD3+ T cells, mast cells, and neutrophils appeared as major sources of IL-17A representing 45%, 4.6%, and 13% of the dermal infiltrate with 40%, 45%, and 35% of cells producing IL-17A, respectively. Finally, by RT-PCR of a large panel of IL-17-related mediators in perilesional skin biopsies of BP patients (n=15), and site-, age, and sex-matched controls (n=10) compared to controls we found elevated mRNA levels in the BP skin of IL-17A, IL-17F, IL-17RA, IL-17RC, RORt, and various IL-17 related cytokines (IL-22, IL-23, IL-21, CCL20, CCR6, IL-23R, and CXCL2, whereas mRNA levels of STAT3, CX3CL1 and CCL2 were significantly decreased. These data indicate that IL-17A and IL-17-related inflammatory mediators may be important in the pathophysiology of BP. Further studies will explore their pathogenic relevance in experimental models of BP in vitro and in vivo.

P065 (OP01/04) | IL-17E favors neutrophil recruitment in psoriasis by activating M2 macrophages to produce IL-8 in a p38 dependent manner

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Background: Psoriasis vulgaris is a chronic recurrent inflammatory skin disease, affecting approximately 2% of the population. We have recently found that IL-17E (also known as IL-25), a member of the IL-17 cytokine family, is over-expressed in lesional psoriatic skin when compared to non lesional and healthy donors. Within the psoriatic plaque, macrophages infiltrating the dermis internalize IL-17E in a receptor induced clathrin-mediated mechanism.

Objective: In this study we investigated the biological effects of IL-17E on macrophages in psoriasis.

Methods: Biopsies were taken from lesional (n=10) and non-lesional psoriatic skin (n=7), biopsies from normal human skin (n=7) served as controls. The number of IL-17E positive cells and the expression of M1/M2 macrophage markers were assessed by immunofluorescence. Macrophages were generated by blood-derived monocytes and tested for their ability to respond to IL-17E. Levels of inflammatory cytokines and chemokines in IL-17E-treated macrophages were determined by qPCR and ELISA. Pharmacological inhibitors were used to dissect the signaling pathways involved. Neutrophils were isolated by percoll gradient method and tested for their ability to migrate in response to IL-8 or macrophage supernatants in chemotaxis transwell assay.

Results: M2-polarized macrophages, but not M1, expressed high level of the IL-17E-specific receptor subunit IL-17RB and responded to IL-17E by producing inflammatory cytokines (such as TNF and IL-6) and chemokines (such as IL-8 and MCP-1) typically expressed by M1 cells. Nuclear factor-kappa B (NF- B), p38 and STAT3 were required for generating the IL-17E-dependent effects. Of note, IL-17E did not stimulate the production of cytokines/chemokines involved in T cell polarization and recruitment. Supernatants of IL-17E-stimulated macrophages contained high levels of IL-8 and favored the attraction of neutrophils to a higher extent compared to supernatants of resting macrophages. Of interest, p38 inhibition impaired IL-8 production and neutrophil chemotaxis. In vivo, intra-dermal injection of rmIL-17E in C57BL/6 mice induced severe dermal inflammation with neutrophil and eosinophil infiltration, in addition to a hyperplastic epidermis, compared to the PBS control group. Consistent with the in vivo and in vitro data, IL-17E+ macrophages possessed a mixed M1/M2 phenotype ex vivo, being positive for both CLEC5A and CD163L1 markers, and the number of IL-17E+ cells in lesional skin correlated with the number of neutrophils, while being inversely proportional to the number of infiltrating T cells.

CLINICAL RESEARCH

P066 | Integrated safety of ixekizumab in patients with moderate to severe psoriasis: Results from a pooled analysis of seven clinical trials

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Background & Objectives: In moderate-to severe psoriasis, long-term treatment is usually required to achieve adequate control of disease activity. This publication analyzes the safety of ixekizumab (IXE), a monoclonal anti-IL-17A antibody with a high binding affinity, which is currently in development for the treatment of psoriasis.

Methods: Treatment-emergent adverse event (TEAE) and serious adverse event (SAE) data were integrated from the induction period of three randomized, controlled trials [RCTs] (0-12 weeks), the maintenance period of 2 of the 3 RCTs with a randomized withdrawal design (12-60 weeks), and all patients exposed to IXE from all seven psoriasis trials (controlled and uncontrolled). For the induction period, patients with moderate to severe psoriasis were randomized to IXE every 2 (IXE Q2W; N=1167) or 4 weeks (IXE Q4W; N=1161) after a 160-mg starting dose, etanercept (ETN) (50 mg biweekly; N=739), or placebo (PBO) (N=791). The maintenance period included IXE-treated patients who had an sPGA 0.1 at Week 12 (responders) who then were re-randomized to IXE Q4W (N=416), IXE every 12 weeks (IXE Q12W, N=408), or PBO/withdrawal group (N=402). The group of all patients exposed to IXE (N=4209), accounted for 6480 patient years (PY) of exposure. Comparison of induction and maintenance periods was descriptive.

Results: During the induction period, the frequency of any TEAE was higher in Total IXE (58.6%), IXE Q2W (58.4%), IXE Q4W (58.8%), and ETN (54.0%) compared to PBO (46.8%). Most TEAEs were mild or moderate. The frequency of AEs reported as severe, SAEs, and discontinuations due to AEs did not differ among treatment groups. During the maintenance period, the exposure-adjusted incidence rate (IR—per hundred patient years) of TEAEs was lower for IXE Q4W patients than for the PBO/withdrawal group (IR: PBO, 123.8; IXE Q12W, 106.2; IXE Q4W, 95.6), with no significant difference observed between the IXE Q12W and IXE Q4W groups. The IR of TEAEs was lower during the maintenance phase than during the induction phase among patients who received continued dosing on IXE Q4W (99.3 and 256.8, respectively). Among all patients exposed to IXE, the exposure adjusted IR of TEAEs was 54.4. Most TEAEs were mild or moderate.

Conclusions: IXE had a safety profile that was similar to ETN during the induction period. The overall incidence of AEs in the Q2W and Q4W dosing regimens were similar. The IR for AEs decreased over time with continued IXE treatment.

P067 | Direct modulation of the skin microbiome as new experimental tool for skin diseases

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With the advent of NGS technologies scientists gained unprecedented insights into the bacterial communities living on our skin. Recent research shows that the cutaneous microbiome plays a crucial role in skin diseases including acne vulgaris. However, so far most investigations yield largely descriptive data. In order to understand pathogenic relationships and pave the way for therapeutic interventions, heterologous skin bacteria have to be established on the skin that alter the composition of the skin microbiome. This raises the question whether a modulated microbiome will be tolerated by the host immune system in the absence of an inflammatory reaction. To answer this question we conducted a prospective clinical study. In this prospective clinical study we applied different bacterial mixtures isolated from several healthy donors on the skin of healthy volunteers and investigated the safety aspects of the resulting modulation of the skin microbiome using routine clinical assessment. We used different mixtures and concentrations of bacteria on three consecutive days after a disinfection treatment. Our data show that a modulation of the skin microbiome at the strain level on healthy skin was well tolerated. Neither skin irritation nor other adverse events were observed in 18 healthy volunteers throughout the study. In a subsequent study we assessed the safety of our method on irritated skin. For this we modulated the skin microbiome of acne vulgaris patients (Leeds score 1, 5-4). Again neither skin irritation nor other adverse events were observed. Our data provide evidence that the composition of the skin microbiome can be safely modulated at the strain level without causing clinical signs of irritation. This opens up new avenues to exploit skin microbiome modulation as a novel experimental and therapeutic tool.

P068 | Cold atmospheric pressure plasma for chronic wound healing

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During the last decades the development of cold atmospheric pressure plasmas led to a new treatment option to heal chronic wounds. Cold plasma consists of partially ionized gas and contains a range of

reactive oxygen and nitrogen species (ROS/RNS) combined with UV radiation and mild heat. Those biologically active components can be modulated and controlled in order to achieve both bactericidal effects and promoting skin regeneration.

Nowadays first cold plasma sources are certified medical devices for the treatment of chronic wounds. Here, we present first data of successful plasma treated type 2 diabetes patients with chronic leg ulcers. The aim of this study was to investigate the impact of cold plasma on 1) wound size and 2) wound exudates for microbial load and content of cytokines of these chronic leg ulcers in order to disentangle the underlying mechanisms of plasma cell interactions.

Following cold plasma application granulation was fostered as well as skin cell proliferation occurred which finally led to a reduction of wound size. However, the most promising fact is that cold plasma was able to kill also multi-resistant bacteria. Successful wound healing is greatly supported by reduction of bacterial load as well as molecular proofs of eukaryotic cell stimulation as detected by Ki67-based histological staining. In addition, determination of cytokine expression pattern revealed a positive influence of cold plasmas on wounds which failed to heal with conventional treatments.

Altogether, cold plasma promotes wound healing in diabetic patients suffering incurable chronic wounds for several weeks to months. Here we demonstrate that cold plasma treatment of such wounds can stimulate cellular activities resulting in a short term activation of cell proliferation. Therefore, cold plasma is a promising tool in chronic wound treatment. However, there is the need to understand the processes of ROS/RNS generation in order to find the balance between activating human skin cells and killing microorganisms. Therefore, much effort has to be done in future studies in order to modulate biological activities and optimizing the plasma treatment on an individual patient level.

P069 (OP04/01) | A novel preclinical model of organotypic slice cultures for pharmacodynamic profiling of human melanomas

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With the growing number of developing and approved targeted therapies and immunotherapies the algorithms of therapy for stage III and IV patients become increasingly complex. A putative response to a selected therapy is mainly influenced by cancer genetics. However, the present (and basically completed) classification of genetic melanoma subtypes does not suffice for a reliable prediction of the quality and the quantity of drug efficacy including the duration of the response until resistance occurs. It is well accepted that further characteristics like epigenetic mechanisms, the tumor stroma and the status of the immune system of the patient play decisive roles for the success of a given cancer therapy. This holds on to be especially true for patients with BRAF wild-type tumors. This population comprises approximately 50% of the melanoma patients which can be genetically subdivided into NRAS mutated melanomas, NF-1 (neurofibromin 1) mutated melanomas and others harboring low frequency mutations. So far there is no approved targeted therapy for these patients with such BRAF wild-type melanomas. However, there is evidence that the classical melanoma driver pathways, the MAPK and PI3K/mTOR signaling pathways, are equally important in the pathogenesis of these tumors compared to BRAF mutated melanomas.

Thus, the prediction of drug response for WT melanoma patients remains a major challenge in the clinic. The response to signal transduction inhibitors and therapy efficacy is determined not only by properties of the drug target but also by concomitant mutations in other signaling molecules and the tumor microenvironment. Therefore, a solid and fast functional test system that preserves melanoma microenvironment and tumor heterogeneity is of great interest.

We have established a reproducible and rapid ex vivo personalized culture method that allows the investigation of anti-tumoral and pharmacological properties of drug combinations (Opti-MIS: Optimized melanoma in vitro slice cultures for preclinical and personalized drug testing). Melanoma punch biopsies or patient derived xenograft tumors were used for the preparation of 400 µm thin tissue slices using a vibratome. The slices were cultivated for five days and treated for four days with clinical relevant drugs like BRAF or MEK inhibitors before measuring tissue viability by an enzymatic assay. Tissue slices were further used for immunohistochemical evaluation of proliferation (Ki67) and apoptosis induction (cleaved PARP). The results were correlated to the genetic background of the tumor and the clinical data of the patient. Our results show that this slice culture model preserves tissue 3D architecture, cell viability and pathway activity up to 5 days ex vivo. Treatment of melanoma slice cultures with inhibitors reduced tissue viability in a reproducible manner and correlated with clinical efficiency and underlying resistant mechanisms. Effects of the drugs on tumor cell proliferation and apoptosis were successfully determined by Ki67 and cleaved PARP stainings.

This new preclinical tissue culture model can be used in a reproducible manner to evaluate the effects of different small molecule inhibitors directly on patient tissue and can help to develop the best therapy option for each patient.

P070 | Rapid onset of efficacy in patients with psoriasis treated with ixekizumab: A pooled analysis of data from two phase 3 randomized clinical trials (UNCOVER-2 and UNCOVER-3)

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Background & Objectives: For patients with psoriasis, rapid onset of clinical improvement is one of the most important attributes of treatment success [1]. In addition, it has been demonstrated that clinical improvement observed early during treatment has predictive value for subsequent clinical response at later time points [2]. In this analysis, we evaluated the speed of onset of clinical improvement in psoriasis patients treated with ixekizumab (anti-IL-17A IgG4 monoclonal antibody with high binding affinity; IXE) compared with placebo and the active comparator, etanercept (ETN).

Methods: Combining data from the 12-week Induction Phase of UNCOVER-2 and UNCOVER-3, 2570 patients with moderate-to-severe plaque psoriasis were randomized to receive placebo (PBO, n=361), high-dose ETN (50 mg bi-weekly; n=740), or a single 80-mg subcutaneous injection of IXE once every 2 weeks (IXE Q2W; n=736) or every 4 weeks (IXE Q4W; n=733) after receiving a 160-mg initial dose at Week 0. Mean percentage improvement was analyzed by MMRM and response rates by Cochran-Mantel-Haenszel test, where missing data were imputed using nonresponse. Time to PASI 75 was estimated using the Kaplan-Meier product limit methodology.

Results: Significant differences in mean % change from baseline (improvement) in the PASI were observed between the IXE treatment groups compared with PBO and ETN as early as Week 1 (P<.001) with mean (SE) % improvements of 32.7 (0.76) in IXE Q2W, 33.6 (0.76) in IXE Q4W, 5.31 (1.08) in PBO, and 10.3 (0.76) in ETN. At Week 2, the mean % improvement was 53.7 (0.86) in IXE Q2W, 53.3 (0.86) in IXE Q4W, 9.25 (1.23) in PBO, and 23.3 (0.86) in ETN. At Week 1, the PASI 50 response rate was 22.8% in the IXE Q2W and 26.6% in IXE Q4W compared with 1.4% in PBO (P<.001) and 3.9% in ETN (P<.001), and at Week 2, the PASI 50 response rate was 58.8% in the IXE Q2W and 57.6% in IXE Q4W compared to 4.2% in PBO (P<.001), and 14.6% in ETN (P<.001). Median time (95% CI) to PASI 75 was 31 (30, 55) days in the IXE Q4W group, 30 (29, 43) days in the IXE Q2W group, and 85 (85, 87) days for the ETN group.

Conclusions: IXE treatment resulted in clinically meaningful improvements (PASI 50) observed as early as Week 1, which were statistically significantly different compared with ETN and PBO. At least 50% of patients had a PASI 75 after approximately 4 weeks of IXE treatment.

1. Seston et al. Arch Dermatol. 2007;143:1175-9
2. Zhu et al. BJD 2013;169:1337-41

P071 | A randomized, double-blind, active- and placebo-controlled phase 3 study of efficacy and safety of ixekizumab, adalimumab, and placebo therapy in patients naïve to biologic disease-modifying antirheumatic drugs with active psoriatic arthritis

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Background: Ixekizumab is a monoclonal antibody under investigation for psoriatic arthritis (PsA) treatment.

Methods: In a phase 3 trial, 417 biologic disease-modifying antirheumatic drug (bDMARD)-naive patients with active PsA were randomized to up to 24 weeks of placebo (N=106); adalimumab 40 mg once every 2 weeks (Q2W; active control; N=101); or ixekizumab 80 mg Q2W (N=103) or once every 4 weeks (Q4W; N=107) following an initial 160 mg dose at Week 0. Endpoints included American College of Rheumatology 20 response (ACR20) at Week 24 (primary), ACR50, ACR70, a 75/90/100% improvement in Psoriasis Area and Severity Index (PASI75/PASI90/PASI100), Disease Activity Score (28 joint count) based on C-reactive protein (DAS28-CRP), Leeds Dactylitis Index (LDI-B), Leeds Enthesitis Index (LEI), and Health Assessment Questionnaire-Disability Index (HAQ-DI) at 12 and 24 weeks, and Van der Heijde modified Total Sharp (mTSS) score at 16 and 24 weeks. Efficacy variables were evaluated using the intent-to-treat population. Continuous data were evaluated using mixed-effects model for repeated measures. Categorical data were compared using a logistic regression model with non-responder imputation for missing values (inadequate responders treated as non-responders).

Results: 382 patients completed 24 weeks; 30.2%, 57.4%, 62.1% and 57.9% of placebo-, adalimumab-, ixekizumab Q2W- and ixekizumab Q4W-treated patients, respectively, had ACR20 responses. At 12 and 24 weeks, a higher percentage of ixekizumab 80 mg Q2W- or Q4W-treated than placebo-treated patients achieved ACR20/50/70 and PASI75/90/100 responses (P≤.001; Week 12 ACR70 not eligible for comparison), and both ixekizumab groups experienced greater reductions than placebo for measures of dactylitis (LDI-B); enthesitis (LEI) reduction in the Q2W group only (Week 12). Disease activity (DAS28-CRP) and functional disability (HAQDI) improved and inhibition of radiographic progression of joint structural damage (mTSS) occurred with both ixekizumab doses compared with placebo (P≤.025). 24- week treatment-emergent adverse events (TEAE) incidence was higher (P≤.025) with ixekizumab and adalimumab compared to placebo; the rate of serious adverse events and discontinuation due to TEAE were similar across groups. No deaths occurred.

Conclusion: In these patients, ixekizumab showed significant, clinically meaningful improvements of disease activity and physical function, reduction in dactylitis, greater skin clearance of plaque psoriasis than placebo, and inhibition of structural progression. Ixekizumab was well tolerated with no unexpected safety findings.

P072 | Treatment of cutaneous mastocytosis—A systematic review

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Background: Mastocytosis is characterized by abnormal growth and accumulation of mast cells in the skin and in extracutaneous organs. The WHO classification of cutaneous mastocytosis includes four types: urticaria pigmentosa, maculopapular cutaneous mastocytosis, diffuse cutaneous mastocytosis and mastocytoma of skin. The prognosis depends on the age of onset and systemic involvement. Histopathology is an essential part of the diagnosis which demonstrates mast cell infiltration of the dermis. Different treatment modalities are used according to clinical findings.

Objectives: To summarize all reported treatments of cutaneous mastocytosis.

Methods: This is a systematic review based on a MEDLINE search of articles in English and German, between 1980 and 2015, to summarize the treatment of cutaneous mastocytosis.

Results: Most medical literature on treatment of cutaneous mastocytosis is limited to individual case reports and small series of patients. Conventional therapeutic options include antihistamines, mast cell stabilizers, topical corticosteroids, photo- and photochemotherapy, but remissions with some other modalities like omalizumab or laser surgery were reported as well.

Conclusions: There are no existing specific criteria for the selection of treatment options in CM. None of the described modalities was approved for first-line therapy. The age of the patient, size, localization of the skin lesions and the related diseases influence the choice of treatment. Treatment of CM should be individualized for each patient considering the type of CM and reduction of risk of anaphylaxis.

P073 | The pivotal role of wide local excision for the treatment of severe hidradenitis suppurativa (Hurley grade III)—Retrospective analyses of 74 patients

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Hidradenitis suppurativa (HS) is a painful, chronic, recurrent inflammatory skin disease that affects terminal hair follicles and apocrine glands. It develops in early adolescence, and is confined to axillary and inguinogenital/gluteal regions. HS affects up to 4% of the general population.

This study aimed to measure the impact of surgery on the individual quality of life in severe grade HS patients. Additionally, parameters such as disease duration, previous therapeutic interventions, postoperative complications (pain, infection, scarring/keloids, wound healing deficiency, mobility restrictions), postoperative recurrence and satisfaction with the cosmetic results were evaluated.

Data from 74 patients (40 male, 34 female) with HS Hurley grad III treated with wide local excision and secondary wound healing were evaluated. Most patients had inguinogenital/gluteal disease (n=51, 68.9%, $P<.001$). Inguinogenital/gluteal disease was pronounced in female patients ($P=.009$). Involvement of both, axillary and inguinogenital/gluteal areas were pronounced in male patients ($P=.018$). Most patients (n=53; 71.6%) had a disease history of more than 5 years at the time of initial presentation at our institution. Wide local excision improved the Dermatology Life Quality Index (DLQI) scores from initially 27.89 (range 2-30; SD=5.3) to 5.31 (range 0-26; SD 7.38; $P<.001$) independent of localization ($P=.195$). 47.3% of patients had postoperative complications, most frequently pain and scarring. Local recurrence rates were calculated with 18.9% from follow-up data covering a period of up to 14 years. 70.3% of patients were highly satisfied with the cosmetic results.

From our study we conclude that wide local excision of affected skin significantly improves the quality of life of HS Hurley grad III patients and has low rates of local recurrence. Further, surgery has the potential to locally heal HS areas. Satisfaction with the cosmetic results is high. The socio-economic footprint of wide local excision, to date, favors surgery over systemic therapies with anti-inflammatory biologics.

P074 | IgE-specific immunoadsorption for treatment of severe atopic dermatitis

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Atopic dermatitis (AD) is a chronic skin disease that causes itchy, red, swollen, and cracked skin. Currently available therapeutic options run from basic emollients, to external and internal drugs as well as light therapy. But for patients with a severe form of the disease therapeutic demand remains still considerably high. Such patients typically show greatly elevated levels of immunoglobulin E (IgE). Since the role of IgE in the pathogenesis of AD is not completely understood, we now assessed the effects of IgE-specific immunoadsorption (IA) on disease activity and quality of life in severe AD. Until now we analyzed 10 patients (age range 17-55 years) that received IgE-specific IA. All of them had previously received treatments according to guideline recommendations without sufficient control of disease and suffered from severe AD for years, with score-points ranging from 34 to 95 (SCORAD) and from 9.8 to 61.2 (EASI). All patients had to complete questionnaires about quality of life (DLQI and "well-being five"). All these parameters, including blood samples and photo documentation, were collected before each cycle, 3-4 weeks after, and 3-4 months after therapy. All patients received a total of five 2-day biweekly treatment cycles of IgE-specific IA for a period of 8 weeks. All patients had highly elevated baseline serum IgE levels (range 1149-23 553 kU/l). The mean reduction of circulating IgE for all five cycles of all 10 patients was 81% (range 67-90%). Interestingly, not only total IgE levels were affected, but antigen-specific IgE directly after IA was significantly reduced as well. However, within the treatment-free interval, both total and antigen-specific IgE levels significantly increased again.

Disease activity after all five cycles was strongly reduced in all 10 patients (mean 39%, range 1-69% as assessed by SCORAD, and mean 59%, range 14-89% in EASI). The quality of life was improved by 47% (DLQI, range 3-90%) and by 162% ("well-being five", range 9-650%). In summary, our results indicate a dramatic and robust reduction of the total and antigen-specific IgE level in serum during IA and a concomitant reduction of disease activity and improvement of quality of life in all 10 patients with AD. Future studies with more patients and a controlled setting are required to confirm these findings.

P075 | Eosinophilia during immunotherapy of metastatic melanoma—A warning signal for possible side effects?

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Immunotherapy with the CTLA-4 antibody ipilimumab and PD-1 antibodies nivolumab and pembrolizumab have revolutionized therapy of metastatic melanoma regarding response rate, progression free and overall survival but also occurrence of high grade immune related adverse events (irAEs), compared to chemotherapy. A newer concept combining ipilimumab and nivolumab has proven to further enhance response rates, yet again raising the occurrence of treatment related high grade irAEs to 55% in a randomized phase 3 study. This is more than three times the rate of nivolumab alone and two times the rate of ipilimumab alone.

In nearly 30% the irAEs led to discontinuation of therapy and impaired the quality of life of late-stage cancer patients. Therefore, management of irAEs by early recognition and early steroid treatment is an increasingly important factor in the treatment of metastatic melanoma.

It had been previously shown that eosinophils can be used as prognostic biomarkers in the treatment with ipilimumab and also pembrolizumab.

Thus, we investigated whether eosinophils can be considered an early marker for irAEs. So far we have registered 64 patients receiving immunotherapy due to metastatic melanoma, of which 69% experienced irAEs of any grade.

Preliminary data shows that of 39 patients (61%) experiencing eosinophilia (>4%) in general, 28 (72%) also experienced irAEs.

Looking for a possible early marker indicating the onset of a new irAE, we analyzed the occurrence of eosinophilia in temporal context to the first irAE for every patient experiencing irAEs. For >60% of our patients eosinophilia was measured before the first irAE was noticed.

We are currently investigating additional possible markers.

Investigating 64 patients with 729 observed cycles of therapy, our cohort is yet too small to make statistically significant statements. However, our results give reason to believe that eosinophils correlate with irAEs and we are confident that further investigation will show how eosinophil aberration can be used as a red flag for upcoming irAEs. Additionally, these insights can then be used in a predictive model to achieve early diagnosis and treatment of irAEs, thus reduce pauses and abortion of therapy as well as significantly increase life quality of the patient.

P076 (OP05/03) | The combination of BRAFi and MEKi: A treatment option for BRAF WT patients?

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15-25% of all melanomas harbor activating NRAS mutations. Activated NRAS stimulates a number of intracellular signaling pathways including the RAF/MEK/ERK pathway. Overall survival for NRAS-mutant melanoma patients is worse than for their wild-type counterparts. In a phase 2 trial, the MEK inhibitor binimetinib showed activity in patients with NRAS-mutant melanoma with overall response rates of >20% and a median progression-free survival of 4 month. In a previous study, we showed that vemurafenib induces apoptosis in BRAFV600-mutant melanoma cells through a mechanism involving induction of endoplasmic reticulum stress (ER). ER stress induction appeared to be an off-target effect of vemurafenib that remarkably enhances its pro-apoptotic activity in BRAFV600-mutant melanoma. In this study, we investigated whether it is possible to take advantage of ER stress induction to enhance the antitumor activity of MEK inhibitors in patients with NRAS-mutant melanoma.

BRAF-mutant and NRAS-mutant metastatic melanoma cell lines were treated with the BRAF inhibitors vemurafenib, dabrafenib and encorafenib, and were subjected to electron microscopy. All the three substances were able to induce morphological features of ER stress, including a significant dilation of the ER in both BRAF-mutant and NRAS-mutant melanoma cell lines. As expected, the BRAF inhibitors inhibited phosphorylation of ERK and growth and induced apoptosis in BRAF-mutant but not in NRAS-mutant melanoma cells. However, encorafenib significantly enhanced growth inhibition and apoptosis induced by the MEK inhibitor binimetinib in NRAS-mutant melanoma cells in monolayer, spheroid and organotypic culture. However, the BRAF inhibitors significantly enhanced growth inhibition and apoptosis induced by MEK inhibitors. Moreover, the expression of the ER stress-related factors ATF4, CHOP and NUPR1 was induced and siRNA inhibition of ATF4 reduced melanoma cell apoptosis induced by the combinational therapy, pointing out its importance. Both ER stress inducers and MEK inhibitors have been reported to increase the abundance of the proapoptotic protein Bim. We addressed the question if the protein content of Bim is upregulated contributing to induction of apoptosis in melanoma cells. Indeed, binimetinib alone and in combination with encorafenib strongly increased the expression of the three described Bim isoforms BimS (12 kDa), BimL (15 kDa), and BimEL (23 kDa) in NRAS-mutated melanoma cells while encorafenib

showed no effect on the protein level of the latter isoforms. Bim knockdown by siRNA led also to a significant impairment of apoptosis rates caused by encorafenib and binimetinib demonstrating the relevance of Bim in apoptosis induction by this combination.

These data demonstrate that in NRAS-mutant melanoma cells the antitumor activity of the MEK inhibitor binimetinib is significantly potentiated by the BRAF inhibitor encorafenib through ER stress induction leading to significant melanoma cell apoptosis. This study provides a strong rationale for clinical evaluation of MEK inhibitors and ER stress inducing BRAF inhibitors in patients with NRAS-mutant rapidly progressing melanoma.

P077 | Patient-centered aspects of dermatological care for patients with psoriasis vulgaris, urticaria or lupus erythematodes

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Introduction & Objectives: An adequate diagnosis and treatment of psoriasis, urticaria or lupus erythematodes is often problematic due to the diverse and complex organ manifestations as well as a wide variety of available therapies. Adequate diagnosis and appropriate treatments of these diseases are often conducted by various medical specialists in both outpatient and in-patient care. Lack of access and ability of the rural population to get an appropriate therapy may lead by these patients to false diagnosis or a therapeutic oversupply. Furthermore, the partly chronic character in the above mentioned diseases with recurrent symptoms, connected with prolonged itching periods and long-lasting treatments can lead to both emotional, mental stress and physical isolation. This situation does not only affect the health but also the overall quality of life of these patients. Therefore, this study addresses the major factors underlying the patient's perceived health status.

Material & Methods: This study is based on a cross-sectional design. Questionnaire administration and clinical documentation takes place when patients were present at the outpatient clinic of the Department of Dermatology, University Hospital Regensburg.

Key inclusion criteria: diagnoses psoriasis, urticaria or lupus erythematodes.

Enrollment: 150 [anticipated], 50 per disease

Number of Groups/Cohorts: 3

Questionnaires: a) DERMATOLOGY LIFE QUALITY INDEX (DLQI) b) EQ-5D-5L

The questionnaires address skin-specific aspects (itching, emotional and behavioral impairments due to skin disease) as well as general aspects (activities of daily living, overall health-status) of quality of life.

Results: Currently recruitment of patients takes place. A first evaluation of n=136 patients out of 150 anticipated showed, that the distribution of the diagnoses were as follows: 49% psoriasis (n=67), 30% urticaria (n=41) and 21% lupus (n=28). 56% of the patients were classified Fitzpatrick skin type III, 33% type II, 6% type I and 5% type 4. The DLQI score was 8.3 by patients with lupus, 9.2 by patients with psoriasis, and 10.8 for patients with urticaria. From these 136 patients, 49% had a journey distance of >50 km. The EQ-5D-5L showed a score of 61.6 for patients with a journey distance of >50 km and 66.3 for patients with a journey distance less than 50 km. 89% of all patients feel it very important that they are being treated by a medical specialist, here especially by a dermatologist. This was more related to patients with a journey distance of >50 km.

Conclusion: This study addresses key factors concerning the patient's perceived health status and serves for the detection of psychosocial burden of the mentioned skin diseases, thus gaining an increasing importance for prospective economic issues. Patients with an access road >50 km, the quality of life was poorer. The majority of patients consider it necessary to consult a medical specialist (here: dermatologist). Overall the study results may help in improving patient care and designing further clinical trials in the field.

P078 | Long-term safety and tolerability of apremilast in patients with moderate to severe psoriasis and cardiometabolic comorbidities: Pooled safety analysis for 156 weeks and beyond from phase 3, randomized, controlled trials (ESTEEM)

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Introduction & Objectives: Psoriasis is associated with an increased risk for comorbidities including metabolic syndrome and cardiovascular disease. Apremilast is an oral PDE4 inhibitor shown to be effective in phase 3, randomized, placebo (PBO)-controlled trials assessing treatment of moderate to severe plaque psoriasis (ESTEEM 1 and 2). We report safety and tolerability of apremilast 30 mg BID (APR) for ≥156 weeks in patients (pts) with and without select cardiometabolic comorbidities in a pooled analysis of the ESTEEM studies.

Materials & Methods: Pts were stratified by presence of ≥1 cardiometabolic comorbidity, defined as glucose metabolism disorder/diabetes, lipid metabolism disorder, BMI ≥30 kg/m^2, and hypertension. Safety findings are reported for 0 to 16 weeks, 0 to ≤52 weeks, and the overall APR-exposure period (0 to ≥156 weeks), which included all pts who received APR regardless of when APR was initiated through February 2015; ~20% of pts received >3 years (>156 weeks) of APR exposure.

Results: Of the 1250 pts included in the analysis for 0 to 16 weeks, most had ≥1 cardiometabolic comorbidity at baseline (PBO: 295/418 [70.6%]; APR: 563/832 [67.7%]). A total of 1184 (cardiometabolic comorbidities: n=811; no cardiometabolic comorbidities: n=373) pts

received APR during 0 to ≤52 weeks and the APR-exposure period. During 0 to 16 weeks, AEs in ≥5% of pts with and without cardiometabolic comorbidities were consistent with the known safety profile of apremilast. The incidence and nature of these AEs were similar between pts with and without cardiometabolic comorbidities in the APR group. During 0 to 16 weeks, incidences of serious AEs were low in pts receiving APR, with and without cardiometabolic comorbidities (2.5% and 1.1%, respectively), and comparable to those in the PBO group (2.0% and 3.3%, respectively); rates remained comparable between pts with and without cardiometabolic comorbidities during 0 to ≤52 weeks (5.2% and 4.3%, respectively) and the APR-exposure period (9.9% and 7.0%, respectively). Similarly, rates of study drug discontinuation due to AEs were low in pts with and without cardiometabolic comorbidities during 0 to 16 weeks (PBO: 4.4% and 1.6%; APR: 5.7% and 4.8%); rates remained comparable during 0 to ≤52 weeks (7.4% and 8.0%, respectively) and the APR-exposure period (11.2% and 10.7%, respectively). The incidence (exposure-adjusted incidence rate/100 pts-yrs) for major adverse cardiac events (MACE) was low with APR in pts with and without cardiometabolic comorbidities (0.6 and 0.0, respectively) during 0 to 16 weeks. The incidence of MACE did not increase during 0 to ≤52 weeks (cardiometabolic comorbidities: 0.6; no cardiometabolic comorbidities: 0.0) and remained low with prolonged exposure to APR (cardiometabolic comorbidities: 0.7; no cardiometabolic comorbidities: 0.2).

Conclusion: APR demonstrated an acceptable safety profile with prolonged exposure for ≥156 weeks in pts with moderate to severe plaque psoriasis and cardiometabolic comorbidities.

DERMATO-ENDOCRINOLOGY

P079 | Topical application of WOL074-009, WOL074-019 and WOL074-029 tripeptides exhibits strong anti-inflammatory activity in a mouse model of psoriasis

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The tripeptide KdPT has broad anti-inflammatory and immunomodulatory activities and it has been shown to be effective in different murine models from intestinal inflammation to psoriasis. Unfortunately, due to its unfavorable physicochemical properties KdPT cannot be developed in a topical formulation. Therefore, we designed and synthesized analogues of KdPT with optimized physicochemical properties. The anti-inflammatory capacities of WOL074-009 (9), WOL074-019 (19), and WOL074-029 (29) were comparable to KdPT in vitro. Hence, these three peptides were selected for in vivo studies.

To characterize the anti-inflammatory and immunomodulatory potential of substance 9, 19 and 29 in vivo we used the mouse model of imiquimod-induced psoriasis-like skin inflammation. The Peptides were injected intravenously (i.v., 5 µg/mouse) into mice with established skin inflammation (days 5 and 6 after the start of imiquimod treatment). Mice treated with either PBS, betamethasone dipropionate (BMDP) or KdPT served as controls. Injection of 9, 19 and 29 significantly ameliorated ongoing skin inflammation as shown by the reduced epidermal thickness, markedly decreased levels of Th1 and Th17 cells in regional lymph nodes as well as lesional skin, and the down-regulated levels of pro-inflammatory cytokines like IL-1beta, IL-6, TNF-alpha, IL-36 or IL-23. Notably, the anti-inflammatory properties of 9 and 19 were comparable to those of KdPT whereas 29 showed an improved anti-inflammatory potential as compared to KdPT. Next, we investigated whether local application of 9, 19 or 29 might be sufficient to ameliorate ongoing imiquimod-induced psoriasis-like skin inflammation. Therefore, mice were topically treated with a vehicle cream or a cream containing 1% emulsified substance 9, 19 or 29 at days 5, 6 and 7 after the start of imiquimod application. Mice locally treated with 9, 19 and 29 showed a significant amelioration of skin inflammation, as demonstrated by the reduction in epidermal thickness, the decreased activation of effector cells and the downregulated expression of pro-inflammatory cytokines in lesional skin. Interestingly, topical treatment was as effective as i.v. application of the compounds. In summary, these data show that 9, 19 and 29, similar to the original tripeptide KdPT, are able to efficiently ameliorate ongoing inflammation in the skin. Because of the improved physicochemical properties 9, 19 and 29 may be formulated for topical application. Furthermore, two compounds show similar, and one (29) even an enhanced efficacy compared to systemically applied KdPT or BMDP.

P080 | Molecular pathogenesis of hidradenitis suppurativa/acne inversa

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Hidradenitis suppurativa/acne inversa (HS) is a chronic inflammatory skin disease of the hair follicles, classically of the intertriginous areas. HS has been associated with smoking, obesity, increased risk of metabolic syndrome and a variety of comorbid diseases, including inflammatory bowel diseases, spondyloarthropathy and cardiovascular disorders. The goal of our project was to elucidate the pathogenesis of HS, identify signaling pathways responsible for the initiation of the disease and to highlight any specific biomarkers which could act as new targets for treatment. Whole genome gene profiling was employed in lesional and healthy skin obtained from European Caucasian female HS patients (mean age 37.4 8.5 years [n=8]) using the Agilent array platform. Confirmation of gene regulation was performed by real-time PCR and immunohistochemistry. The study was approved by the Ethics Committee of the Charité – Universitaetsmedizin Berlin and was conducted according to the Helsinki Declaration. Amongst 1186

differentially regulated genes 704 showed an upregulation and 482 a downregulation in involved vs healthy skin of HS patients. Significantly regulated signaling pathways which may play a key role in the pathogenesis of HS were atherosclerosis signaling, the LXR/RXR pathway, aryl hydrocarbon receptor signaling, fatty acid oxidation, the crosstalk between dendritic cells and natural killer cells, retinol biosynthesis, protein ubiquitination, IL-4, IL-6, IL-8, IL-10, IL-12, IL-15, IL-17 signaling, CREB signaling and tight junction signaling. Potential positive disease markers were detected, such as PI3, S100A9, S100A7, SPRR3 as well as potential positive activity markers, such as SERPINB3, SERPINB4 and CK6B. Our data provide first evidence on the gene pathways in HS and highlight the major role of inflammation, metabolic and environmental factors in the pathogenesis of the disease.

P081 (OP06/05) | Insights into the mechanism of action of insulin-like growth factor-1 and insulin in human T cells in vitro

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Acne is presenting as a chronic and relapsing inflammatory disorder. Inflammation and adaptive immune responses play an important role in all stages of this disorder. Regular T cell trafficking has been detected around normal follicles in biopsies from acne patients, however, around clinically noninflamed follicles the number was already significantly increased. Accordingly, T cells contribute to the initiation of inflammation in acne. It has been reported that hyperglycemic food increases insulin-like growth factor 1 (IGF-1) and insulin signaling and regulates endocrine responses and thereby may modulate the course of acne. Our previous study showed that 1.0 μM IGF-1 and insulin activate the phosphoinositide 3-kinase (PI3K)/Akt/forkhead box-O1 (FoxO1) pathway in human SZ95 sebocytes. The aim of our present study was to investigate the role of IGF-1 and insulin as putative acnegenic stimuli on the PI3K/Akt/FoxO1 pathway in human primary T cells and on the molecular functions of T cells in vitro. T cells were activated with CD3 antibody and then stimulated with 0.001 μM IGF-1 and 1.0 μM insulin in the presence or absence of 20 μM PI3K inhibitor LY294002 in a time-dependent manner. Nuclear and cytoplasmic expression of p-Akt, FoxO1, and p-FoxO1 were measured by western blot and fluorescence microscopy and FoxO transcriptional activity was determined by dual luciferase reporter assay. Proliferation of T cells was analyzed by [3H]-thymidine incorporation assay. Expression of toll-like receptor (TLR2/4) was determined by flow cytometry. T cells were also exposed to SZ95 sebocyte supernatants prestimulated with IGF-1 or insulin and PI3K pathway activation and T cell proliferation were explored.

Our results showed that 0.001 μM IGF-1 and 1.0 μM insulin activate the PI3K pathway in CD3-activated T cells leading to up-regulation of p-Akt and p-FoxO1 at 15 and 30 minutes compared to CD3-activated control cells. Nuclear FoxO1 was decreased after 15 and 30 minutes. Furthermore, FoxO transcriptional activity was reduced upon IGF-1 and insulin stimulation at 15 minutes compared to CD3-activated control cells. 0.001 μM IGF-1 and 1 μM insulin increased CD3-activated T cell proliferation after different time points with a maximum increase of 29% at 72 hours. Our data further showed that IGF-1 and insulin have no significant effect on TLR2/4 expression in CD3-activated T cells. Interestingly, supernatants from IGF-1- or insulin-stimulated sebocytes activated the PI3K pathway in T cells with up-regulation of p-Akt and p-FoxO1 at 15 minutes. Furthermore, [3H]-thymidine incorporation assays indicated that supernatants from IGF-1- or insulin-stimulated sebocytes significantly reduce T cell proliferation with a maximum suppression of 50% after 72 hours.

Taken together, this study helps to support the hypothesis from in vitro results that in vivo high glycemic load diet which increases IGF-1 and insulin may contribute to induce activation of the PI3K pathway, reduction of FoxO transcriptional activity, and increase of proliferation in human primary T cells. However, they do not influence TLR expression in T cells. In addition, factors secreted by IGF-1- and insulin-stimulated sebocytes have an ability to induce the PI3K pathway in T cells and they reduce T cell proliferation, which probably can reflect a protective mechanism of the sebaceous gland basal cells.

P082 | Insights into the mechanism of action of isotretinoin in human sebocytes in vitro

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Isotretinoin [13-cis retinoic acid (13-cis RA)] is the most potent treatment against severe acne. However, its molecular mechanism of action has not been completely investigated. Recently, research in cancer focused more on retinoid actions mediated via retinoic acid receptor (RAR)-independent pathways such as extracellular signal-regulated kinase (ERK1/2) and phosphoinositide 3-kinase (PI3K). No study to date has investigated the action of isotretinoin on these pathways in sebocytes. Using SZ95 sebocytes as a model, we investigated the effect of a physiological dose of 0.1 μM isotretinoin on the PI3K/Akt/forkhead box-O1 (FoxO1) pathway and molecular function of sebocytes in the presence or absence of insulin-like growth factor 1 (IGF-1) and insulin. SZ95 sebocytes were treated under light protection with 0.1 μM isotretinoin in the presence or absence of 1.0 and 0.1 μM IGF-1 or insulin or LY294002 inhibitor in a time- dependent manner and expression of p-FoxO1 and p- Akt was analyzed by western blot. FoxO transcriptional activity was measured by dual luciferase assay. Nuclear and cytoplasmic mobilization of FoxO1, p-FoxO1, Akt, and p-Akt were determined by immunofluorescence microscopy.

Proliferation of sebocytes was measured by [3H]-thymidine incorporation assay and differentiation by semiquantitative analysis of lipid droplet accumulation using oil Red o staining.

Our results showed that isotretinoin activates the PI3K/Akt/FoxO1 pathway and reduced the nuclear FoxO1 and FoxO transcriptional activity in untreated sebocytes and IGF-1- and insulin-stimulated sebocytes. Isotretinoin alone and in combination with the LY294002 inhibitor suppressed proliferation of untreated sebocytes and IGF-1- and insulin-stimulated sebocytes. Our results showed that the LY294002 inhibitor cannot restore the suppression of proliferation mediated by isotretinoin. Furthermore, isotretinoin reduced lipogenesis in untreated sebocytes and it normalized the lipid accumulation in IGF-1- and insulin-stimulated sebocytes. The LY294002 inhibitor did not restore the decrease of lipogenesis after isotretinoin treatment

These data demonstrate that isotretinoin in a serum achievable concentration of 0.1 μM activates the PI3K/Akt pathway and reduces the expression of nuclear FoxO1. Isotretinoin suppresses proliferation and lipogenesis of sebocytes however by PI3K-independent mechanisms.

P083 | Tropisetron attenuates the inflammatory response in epidermal keratinocytes and acts anti-inflammatory in a mouse model of psoriasis

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Tropisetron is a serotonin receptor (5-HT-R)-modulating agent and approved as an antiemetic for patients undergoing chemotherapy. Recently, we found that tropisetron not only suppressed collagen synthesis in dermal fibroblasts but also elicited antifibrotic effects in the bleomycin mouse model of scleroderma. Interestingly, this effect of tropisetron was mediated by alpha7 nicotinic acetylcholine receptors (alpha7nAchR) but not by 5-HT-Rs in fibroblasts. As bleomycin-induced fibrosis is considered an inflammation-driven model of experimental fibrosis we investigated whether tropisetron can also affect inflammatory cell responses of human epidermal keratinocytes (NHK) which are key cells in the regulation of skin homeostasis. We could show that tropisetron significantly suppressed tumor necrosis factor (TNF)-alpha-induced mRNA expression of both interleukin (IL)-6 and IL-8 in NHK. Tropisetron did not affect canonical p65/NF-kappaB signalling. Moreover, the anti-inflammatory effect of tropisetron on NHK was neither mediated by 5-HT3-R nor 5-HT4-R since these receptors were undetectable in these cells. In contrast, NHK expressed alpha7nAchR which previously were found to bind tropisetron. In accordance with these findings, the alpha7nAchR antagonist alpha-bungarotoxin neutralized whereas AR-R17779, an alpha7nAchR agonist, mimicked the suppressive effect of tropisetron on TNF-alpha-mediated IL-6 and IL-8 expression in NHK. To prove the in vivo relevance of these in vitro data we used the imiquimod mouse model of psoriasis, an established model of inflammation. Application of tropisetron resulted in significantly reduced levels of genes involved in the inflammatory cell response such as TNF-alpha, IL-17, IL-23 and monocyte chemotactic protein (MCP)1. These in vivo results confirm an anti-inflammatory activity of this agent in experimentally-induced psoriasis. In conclusion, our findings suggest that tropisetron and probably other alpha7nAchR-activating agents could be useful for the future therapy of psoriasis and other inflammatory skin diseases.

P084 | Therapeutic potential of the Nox1/4 inhibitor GKT137831 in scleroderma

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The pathogenesis of systemic sclerosis (SSc) is still incompletely understood and effective therapies are urgently needed. Transforming growth factor-β1 (TGF-beta1)-mediated activation of fibroblasts and oxidative stress are crucially involved in the development of tissue fibrosis. Recently, we could show that Nox4, a member of the 7 nicotinamide adenine dinucleotide phosphate oxidase (Nox) family, is strongly upregulated by TGF-beta1 in normal human fibroblasts (HDFs). In contrast, these cells did neither express Nox1, Nox2 nor Nox5. Genetic silencing of Nox4 as well as inhibition of Nox enzyme activity by the pan-Nox inhibitor diphenyleneiodonium neutralized this effect of TGF-beta1 (Dosoki et al. 2016). Here, we examined the impact of GKT137831, a first-in-class small molecule dual-specific Nox1/4 inhibitor in vitro. GKT137831 did not affect cell viability and metabolic activity of HDFs at doses from 0.1-50 μM as shown by XTT test and crystal violet assay. Next, we investigated whether GKT137831 counteracts the profibrotic impact of TGF-β1 in vitro at RNA and protein levels using real-time RT-PCR analysis, procollagen type I C-terminal peptide (PICP) ELISA and immunofluorescence analysis. Importantly, GKT137831 not only suppressed TGF-β1-mediated mRNA expression of collagen type I, but also induction of both α-smooth muscle actin and fibronectin 1, two established myofibroblast markers at mRNA level. At the protein level, GKT137831 attenuated TGF-β1-induced collagen type I secretion. Further, low concentrations of GKT137831 reduced the protein expression of α-SMA and fibronectin as demonstrated by immunofluorescence analysis. Our findings strongly encourage subsequent in vivo studies employing GKT137831 in various models of experimentally induced fibrosis to assess its clinical potential in fibrotic skin disease.

P085 | UVR-mediated decrease of epidermal proliferation/differentiation is inhibited by melatonin and its metabolites AFMK and AMK

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The largest organ of the human body guaranteeing the barrier to the environment is the skin. It can react to external and internal stimuli via the skin immune, the pigmentary, and the skin endocrine system. Skin is a recognized target for melatonin (MEL, N-acetyl-5-methoxytryptamine) which acts against UV-induced skin damage, regulates follicular growth or melanogenesis, and increases proliferation and differentiation of epidermal keratinocytes. Apart from melatonin itself, its main kynurenic metabolites AFMK (N1-acetyl-N2-formyl-5-methoxykynuramine) and AMK (N1-acetyl-5-methoxykynuramine) have been recently shown to significantly enhance epidermal differentiation of human skin ex vivo, hereby contributing to maintaining skin homeostasis and barrier altered by ultraviolet radiation (UVR). Exposure of the skin to UVR leads to short term responses (erythema, sunburn and suntan) as well as long term effects including photoaging and skin cancer. Here, we investigated the ability of MEL, AFMK and AMK to stabilize skin homeostasis under UVR conditions in human ex vivo full-thickness skin organ culture. Skin was irradiated with the validated UV dose of 300 mJ/cm^2 (UVB/A) or sham-irradiated (0 mJ/cm^2) (control) and pre-incubated with or without MEL, AFMK and AMK (10-3 mol/L) for 1 hour prior to UVR exposure. The skin was then directly removed from the culture (0 hour) or further cultivated for 24 or 48 hours, respectively. Immunofluorescence staining of cryosections of the skin at the specified time points and UV-doses showed that UVR decreased protein expression of insulin like growth factor-1 (IGF-I), cytokeratin-14 (K14), a marker of non-differentiating (proliferating) basal layer keratinocytes, as well as p63 protein, a multi-isoform p53 family member required for epidermal development by 43% (IGF-I; P<.001), 24% (K-14; P<.01) and 49% (p63; P<.001) directly after UVR exposure (0 hour), 40% (IGF-I; P<.001), 22% (K-14; P<.01) and 42% (p63; P<.001) after 24 hours, as well as 42% (IGF-I; P<.001), 12% (K-14; P<.05) and 52% (p63; P<.001) after 48 hours post-UVR, respectively. Pre-incubation with the tested compounds inhibited the negative effects of UVR leading to 12% (MEL; P<.01), 14% (AFMK; P<.01) and 15% (AMK; P<.01) enhancements of IGF-I after 24 hours. Further investigations at the same time point revealed increases by 33% (MEL; P<.01), 37% (AFMK; P<.01) and 47% (AMK; P<.001) for K-14 and 23% (MEL; P<.001), 14% (AFMK; P<.05) and 17% (AMK; P<.05) for p63. At 48 hours post UV exposure, protein levels were increased by 11% (MEL; P<.01), 17% (AFMK; P<.01) and 18% (AMK; P<.01) for IGF-I, by 38% (MEL; P<.001), 37% (AFMK; P<.01) and 23% (AMK; P<.001) for K-14 and by 23% (MEL and AFMK; P<.001), 25% (AMK; P<.001) for p63. Concurrent gene expression analysis showed a similar pattern of regulation compared to protein level. To conclude, melatonin as well as its metabolites are able to maintain structure and integrity of human epidermis and therefore can prominently attenuate alterations in skin homeostasis caused by UVR.

P086 | Effects of extracellular calcium and 1,25 dihydroxyvitamin D3 on sebaceous gland cells in vitro and in vivo

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Calcium and 1,25 dihydroxyvitamin D3 (1,25(OH)2D3)are promoters of epithelial cell functions; however their effects on sebaceous glands are unknown. In this study, morphology, ultrastructure, cell numbers, lipid synthesis and apoptosis of SZ95 sebocytes were assessed in vitro under different concentrations of extracellular calcium with or without 1,25(OH)2D3. Moreover, serum calcium and 1,25(OH)2D3 levels were assessed in acne and non-acne patients (controls). Under conditions of low extracellular calcium, lipogenesis and cell detachment were observed. Increasing extracellular calcium enhanced sebocyte numbers, induced epithelial morphology and reduced lipogenesis. Moreover, a reduction in extracellular calcium reduced E-cadherin and enhanced caspase 3/7 activity (apoptosis), whereas calcium chelation by EGTA (ethylene glycol-bis(β-aminoethyl ether)- N,N,N′,N′-tetraacetic acid) resulted in enhanced lipogenesis. 1,25(OH)2D3 decreased sebaceous lipogenesis, but also induced signs of autophagy. In the clinical study, patients and controls exhibited normal serum calcium levels, whereas younger acne patients presented higher levels than older patients and controls. In addition, younger acne patients presented lower 1,25(OH)2D3 levels than did older ones. In conclusion, extracellular calcium and 1,25(OH)2D3 regulate sebocyte morphology, increase cell numbers, decrease sebaceous lipogenesis and induce cell autophagy in vitro. The increased ionized calcium and the reduced 1,25(OH)2D3 levels detected in the serum of younger patients with acne may contribute respectively to increased sebaceous gland volume and enhanced lipogenesis.

P087 | Zileuton, an efficient and safe systemic anti-acne drug

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Human sebocytes and inflammatory cells express the enzymes of the leukotriene pathway at mRNA and protein levels and enzymes involved in LTB4 biosynthesis are activated in sebaceous glands of acne lesions. Pre-treatment of SZ95 sebocytes with Zileuton, a 5-lipoxygenase inhibitor, partially prevented short-term arachidonic acid (AA)-induced effects, such as enhancement of LTB4 and interleukin (IL)-6 release and increase of neutral lipid content. Long-term treatment with Zileuton directly reduced the content of neutral lipids and IL-6 release from SZ95 sebocytes. In a first pilot clinical study with 10 patients with papulopustular acne Zileuton 4 × 600 mg/d p.o. for 3 months decreased the acne severity index in a time-dependent manner being 41% of the initial score at week 12 (P<.05). This was mostly due to a decrease of the number of inflammatory lesions (−29%, P<.01). In addition, total sebum lipids significantly decreased (−35%, P<.05) and the pro-inflammatory free fatty acids (−22%) and lipoperoxides (−26%) were markedly diminished in patients' sebum under treatment. The magnitude of clinical improvement strongly correlated with the reduction of total sebum lipids

(r^2=.81) and free fatty acids (r^2=.82). In a further study, a 40-year-old female with mild disseminated sebaceous gland hyperplasia and seborrhea, responded similarly under Zileuton over 2 weeks and – after a wash-out phase – low-dose isotretinoin (10 mg/2nd d) over 5 weeks with normalization of casual skin surface lipids and facial sebum synthesis. Finally, a phase II multicenter, clinical study in 101 patients with mild to moderate inflammatory facial acne showed a significant efficacy of Zileuton in a subset of patients with moderate acne, whereas those patients treated with Zileuton (n=26) showed a mean decrease in inflammatory lesions of 41.6% compared to 26.2% in the placebo group (*P*=.025). In all clinical studies, Zileuton was safe and well tolerated. Zileuton, the first genuine anti-inflammatory compound available has been shown effective and safe in the treatment of moderate to severe acne vulgaris and can substitute systemic antibiotic in the armamentarium of acne treatment.

P088 | Actualization of the European S1 guideline for the treatment of hidradenitis suppurativa/acne inversa

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Guidelines for treatment have a validity period, however, partial actualization is required in case that major new findings have occurred. The European S1 guideline for the treatment of hidradenitis suppurativa/acne inversa (HS) was published in 2015 (Zouboulis CC et al. J Eur Acad Dermatol Venereol 29:619-44, 2015). Since the field is developing rapidly, a systematic literature search in the Medline database was conducted for the period 2013-2015 under the term "hidradenitis" in order to evaluate the current validity of the guideline. WE found that no change is required on basic aspects: The European S1 HS guideline suggests that the disease should be treated based on its individual subjective impact and objective severity. Locally recurring lesions can be treated by classical surgery or LASER techniques, whereas medical treatment either as monotherapy or in combination with radical surgery is more appropriate for widely spread lesions. New aspects represent: First line medical therapy may include a combination of systemic antibiotics (clindamycin plus rifampicin) or single systemic antibiotics (tetracycline) and acitretin. As second line, medical treatment with biologics can be administered. The anti-TNF agent adalimumab represents the only approved treatment for moderate to severe HS in adults with an inadequate response to conventional systemic HS treatment. Hurley severity grading is no more sufficient for treatment decision and a new dynamic HS severity score (mild/moderate/severe disease) is required. Weight loss and tobacco abstinence are adjuvant measurements, proven to improve the severity of HS as independent factors. In conclusion, new important findings have emerged since the publication of the European S1 guideline for the treatment of hidradenitis suppurativa/acne inversa, which require a partial guideline actualization.

P089 | Detection of olfactory receptors in various cutaneous cell types in vitro

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Olfactory receptors (ORs) are typically expressed in the nasal epithelium where they mediate communication between environmental odorants and the nervous system. Interestingly, there is recent evidence that expression of these G-protein-coupled receptors occurs also outside olfactory sensory neurons, e. g. in the skin pointing towards a broader function of ORs far beyond smell perception. Accordingly, we could recently show that functional OR2AT4 is expressed by human epidermal keratinocytes. A synthetic sandalwood odorant induced wound-healing processes in human keratinocytes via OR2AT4 (Busse et al. Invest Dermatol 2014; 134: 2823-2832). Here we further investigated the expression of additional ORs, i. e. OR6M1, OR11A1, OR5V1, OR6V1, OR14A2, OR5D16, OR6Y1, OR10J5, OR1G1, OR11H7P, OR2J3, OR5A1 and OR7D4 in cultured human epidermal keratinocytes, epidermal melanocytes and dermal fibroblasts using RT-PCR analysis, Western immunoblotting and immunofluorescence analysis. While OR11A1, OR5V1, OR14A2, OR5D16, OR6Y1, OR10J5 and OR1G1 were undetectable in all examined cell types human epidermal melanocytes as well as epidermal keratinocytes consistently (n=3 per cell type) expressed ORV1 at the RNA level. Western immunoblotting confirmed expression of this OR in both cell types. Moreover, immunofluorescence analysis revealed specific ORV1 immunoreactivity at the cell surface and within the cytoplasm. Further studies are currently underway to extend these in vitro findings in human skin in situ.

DERMATOPATHOLOGY

P090 | Analysis of early inflammatory processes in chemically induced mouse models of dermal fibrosis: pathogenic role of $CD11b^+$ and $Ly6C^+$ myeloid cells

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Systemic sclerosis is a complex and incompletely understood autoimmune disease associated with a production of reactive oxygen species and recruitment of activated immune cells resulting in fibrosis of the skin and various organs like lung and kidney (SSc). In our study we focused on the analysis of the function of myeloid immune cells

in the early phase of cutaneous fibrosis to identify novel targets for innovative therapeutic strategies. We used two established chemically induced mouse models of scleroderma which resemble certain aspects of the human cutaneous scleroderma (Scl). Scl was induced by daily intradermal administration of the cytostatic drug bleomycin which triggers ROS production of endothelial cells indirectly or by hypochloric acid (HOCl) as direct ROS donor. Both models showed a significant increase in dermal thickness after 28 days and a prominent increase in collagen fibers and total collagen levels accompanied by disorganized collagen architecture (H&E, Goldner's trichrome and Sirius Red staining). As a hallmark for profound myofibroblast activation we observed increase numbers of α-SMA$^+$ cells in both models. Flow cytometric analysis of Scl skin demonstrated an early cellular infiltrate after 7 days with significantly increased percentages of activated myeloid cells (CD11b$^+$MHCII$^+$) and migratory inflammatory monocytes (CD11b$^+$Ly6C$^+$). As this phenotype was more pronounced in HOCl mice, we aimed to analyze the functional role of the myeloid CD11b$^+$Ly6C$^+$ cells during the early phase of HOCl-induced Scl by depleting antibodies. To this end mice were injected i.p. every other day with antibodies against CD11b (IgG2b) and Ly6C (IgG2a) (Or isotype control antibodies) respectively resulting in a significant depletion of circulating myeloid cells in the skin and blood until day 5. Scl was simultaneously induced by daily intradermal administration of hypochloric acid (HOCl). The absence of myeloid CD11b$^+$ and Ly6C$^+$ immune cells led to a reduction of HOCl induced skin thickening at days 7 and 14 which became statistically significant at day 28 compared to controls. In conclusion, our study demonstrates that CD11b$^+$ and Ly6C$^+$ myeloid cells play a crucial role in the early phase of Scl and may serve as targets for novel preventive or therapeutic strategies in fibrosis.

P091 (OP04/04) | A novel mouse model for anti-laminin 332 mucous membrane pemphigoid

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Anti-laminin 332 mucous membrane pemphigoid (MMP) is a subepidermal blistering autoimmune dermatosis characterized by autoantibodies against laminin 332, a structural protein of epidermal/epithelial basement membranes. Most patients develop autoantibodies against the alpha 3 chain of laminin 332. Until now, only little data are available about the pathophysiological mechanisms of this disease. Based on the identification of two immunodominant regions of human laminin alpha 3, we established a novel experimental model in adult C57BL/6 mice by the passive transfer of rabbit IgG raised against the murine homologues of these fragments (mLAMα3). After 12 days of repeated s.c. injection of rabbit anti-mLAMα3 IgG, erosions and crusts occurred predominantly around the snout, eyes, and ears. Interestingly, loss of up to 25% body weight was observed and histopathology revealed conjunctival lesions in about 80% of mice. Lesions in the oral mucosa cavity and oesophagus were present in 80% and 16% of mice, respectively, while stomach and colon were not affected. Direct immunofluorescence microscopy showed IgG and C3 deposits at the basal membrane zone of skin, buccal mucosa, tongue, oesophagus, colon, and conjunctiva. Fc gamma chain-deficient mice were completely protected from the pathogenic effect of anti-LAMα3 IgG and C5aR1-deficient mice developed significantly less disease compared to wild-type animals (P<.001). The extensive involvement of conjunctiva and oral mucosa mirrors the human disease and clearly differentiates the novel model from previously established mouse models of bullous pemphigoid and epidermolysis bullosa acquisita. The anti-mLAMα3 IgG-induced mouse model will allow further dissecting the pathomechanisms of the disease and exploring more specific anti-inflammatory mediators for autoantibody-mediated diseases.

P092 | Neutrophil extracellular traps (NETs) are crucial in the pathogenesis of IL-23- mediated psoriasis-like skin inflammation

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Psoriasis is a chronic recurrent systemic inflammatory disease with considerable morbidity. In spite of its clinical importance, the pathogenesis of psoriasis is not fully understood. Of late, neutrophil extracellular traps have been implicated in psoriasis, as these networks of chromatin and antimicrobial peptides, expelled by neutrophilic granulocytes, can be found in psoriatic plaques. Moreover, IL-17, a key player in the signal pathway of psoriasis, co-localizes with NETs within psoriatic skin, suggesting a hitherto unrecognized role for neutrophils and NETs in this disease. Clarifying the pathophysiological role of NETs in psoriasis could provide novel pharmacological targets in psoriasis therapy.

Therefore, we investigated the role of NETs in a murine model of psoriasis, using intradermal injections of IL-23 to induce a psoriasis-like skin phenotype.

During the early phase of inflammation (day 5), NETs were readily found in IL-23- treated skin, as determined by fluorescence microscopy. Interestingly, the propensity of neutrophils isolated from the peripheral blood is not increased, indicating an effect locally restricted to the inflamed skin. However, at day 10, NETs were no longer detectable in the skin sections, suggesting their degradation as the inflammation entered a more chronic phase. Systemic treatment of mice with DNase I, which degrades NETs, efficiently prevented the development of an inflammatory phenotype.

Our results shed new light on the pathophysiological role of neutrophils in psoriasis and suggest that the inhibition of NET formation or their degradation may provide a pharmacological approach to ameliorate or prevent flare-ups in psoriasis patients.

P093 | Mast cells participate in the inflammatory response in bullous pemphigoid

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Pemphigoid diseases comprise a family of autoimmune diseases defined by autoantibodies against proteins of the dermal-epidermal junction. Bullous pemphigoid (BP) is the most common disease and, clinically, presents with dense blisters, erythema and wheals. Mast cells have long been implicated in the pathogenesis of BP. For instance, the mast cell-specific protease tryptase has been linked to initiate the inflammatory response and to induce degradation of the dermal-epidermal junction.

In order to further elucidate the role of mast cells and their mediators in BP, we analyzed numbers of mast cells in skin biopsies of BP patients and scrutinized their activation state (degranulated or not degranulated).

Skin biopsies of patients with BP and control subjects with normal skin were stained by immunofluorescence using an antibody against tryptase. Evaluation of skin sections was performed by two independent observers in a blinded fashion.

Analyzing the number of mast cells, we did not observe a significant difference between BP patients and controls (35.8 5.3 mast cells/visual field in BP; 35.8 2.9 mast cells/visual field in controls; mean SEM). However, there was a significant difference in the state of mast cell degranulation. In BP, 53.5% 3.5 mast cells were degranulated, whereas only 21.6% 3.3 mast cells were degranulated in control subjects. Equally, fewer mast cells (46.5% 3.5) in BP remained not degranulated compared to controls (78.4% 3.3).

With these first data we conclude that mast cells participate in the pathogenesis of BP. Our lab aims to further investigate the functional role of mast cells exploring mouse models of BP in mast cell-transgenic mice.

P094 (OP02/02) | The adaptive response of MSCs on neutrophil activation depends on TRL-4 mediated sensing at the wound site

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Mesenchymal stem cells (MSCs) are multipotent progenitor cells found in a variety of tissues including skin, where they support tissue renewal and homeostasis. MSCs are most likely endowed with the capacity to sense environmental cues like infection and to generate an integrated adaptive response in the interest of tissue protection. So far it is, however, largely unexplored how MSCs sense their environment and how they mount an adaptive response to shape the function and activation state of distinct immune cells in particular neutrophils during tissue injury. While neutrophils are important in initial phases of wound healing to effectively cleanse tissue debris and avoid microbial infections after wounding, their unrestrained activation may lead to tissue break down and delayed wound healing. Previously, we have shown that MSCs suppress neutrophil activation by dampening their oxidative burst, the release of reactive oxygen species (ROS), proteolytic enzymes and by phagocytosis of activated neutrophils leading to scar reduced tissue repair. In the present study, we wished to investigate how MSCs adaptively regulate neutrophils function under conditions of wound infection, where MSC suppression of neutrophil functions would rather be detrimental. To address this question we evaluated the adaptive response of adipose derived MSCs (AD-MSCs) on activated neutrophil functions in the presence and absence of pathogen-associated molecular patterns (PAMP) such as bacterial lipopolysaccharide (LPS) mimicking an infectious wound environment. Of note, LPS-treated AD-MSCs substantially augment neutrophil activation resulting in an increased neutrophil extracellular trap (NET) formation and increased ROS production as opposed to MSCs suppression of activated neutrophils under "noninfectious" conditions. To further explore whether toll like receptor-4 (TLR-4) present on MSCs surface (as assessed by FACS analysis), is involved in the adaptive response of AD-MSC, we specifically silenced the TLR-4 receptor gene employing specific siRNA. Our results show that TLR-4 silenced MSCs upon LPS treatment failed to activate neutrophils, subsequent NET formation and ROS production, indicating a causal role for TLR-4-dependent sensing LPS, subsequent signaling and shaping the adaptive response of AD-MSCs. RNA Seq analysis, RT PCR, antibody arrays, and factor specific ELISA of MSCs (and their supernatants) cultured in the presence or absence of LPS uncovered GCP-2 and IL-8, which are known to recruit and activate neutrophils. Collectively, we identified the mechanism underlying the master role of MSCs in the control of infectious cues and tissue integrity. Our data may even hold promise to be therapeutically exploited for the benefit of patients with difficult-to-treat and/or infected wounds.

EPIDEMIOLOGY

P095 | Off-label prescriptions and decisions on cost coverage requests – a retrospective analysis

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Background: "Off-label use" is defined as the prescription of pharmaceutical products beyond their approved label. Rare diseases often lack in-label treatment options. Reimbursement of off-label therapy

costs is only warranted under specific circumstances. In order to avoid claims for the return of costs (German "regress"), prescribing physicians can apply for cost-coverage by the insurance provider on an individual basis prior to treatment initiation.

Methods: We conducted a chart review of cost-coverage requests from two clinics of a dermatological university outpatient department between 2010-2012 (clinic for autoimmune diseases and urticaria clinic). Insurance providers, acceptance rates, reasons for rejection, and processing times were assessed. The influence of patients' age and drug costs of the suggested treatment were analyzed using the Fisher's exact test.

Results: The analysis showed that 56.8% of the off-label applications (n=44) were approved during the first round. The rate increased to 75.0% when including approvals, which were granted after up to two rejections. The time between initial application and insurers' response was 49 days (median). In case of cost coverage, treatments were initiated 92 days (median) after the initial request. Costs of the suggested therapy and patients' age did not have a statistically significant influence on approval.

Conclusions: The present case series shows that payers agreed to reimburse costs of suggested off-label therapies in the majority of the cases. However, 25.0% were rejected despite repeated requests. In these cases, initiation of the requested treatment would impose the risk of claims for the return of costs by the prescribing physician. The two-month duration until approval of cost-coverage requests poses a relevant problem regarding timely patient care.

P096 | Development and validation of the quality of life questionnaire for cholinergic urticaria: CholU-QoL

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Cholinergic urticaria (CholU) is a frequent form of chronic inducible urticaria where itchy wheal and flare type skin reactions in response to physical exercise or passive warming occur. Although the patients can have a high burden of disease, no disease specific quality of life instrument is available.

In this study, we developed and validated a disease specific quality of life (QoL) instrument for CholU patients, the Cholinergic Urticaria Quality of Life Questionnaire (CholU-QoL).

Using literature search, semi-structured patient interviews, and expert opinion we developed 28 potential ChoU-QoL items. Item selection was performed via impact analysis in 50 patients and a final review for validity. The resulting CholU-QoL was tested for validity, reliability and influence factors in 88 patients. An English version of the CholU-QoL was developed in parallel.

In total, 88 CholU patients were recruited for the CholU-QoL validation study at two German study sites. The final 28-item questionnaire has a 5-domain structure ("symptoms", "functional life", "social interaction", "therapy", "emotions"), a valid total score and good test-retest reliability. Detailed analyses revealed that the CholU-QoL is a robust tool in terms of its test-retest reliability and not influenced by the gender or age of the CholU patients.

The CholU-QoL is the first disease specific QoL instrument for a form of inducible urticaria. This questionnaire resembles a valuable tool for upcoming clinical trials and for routine patient management.

P097 | Validity, reliability and responsiveness of the Urticaria Activity Scores

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Background: Chronic spontaneous urticaria (CSU) is characterized by fluctuating symptoms. Its disease activity is commonly determined by the Urticaria Activity Score (UAS). The UAS is a patient reported activity measure, recommended by the EAACI/ GA2LEN/EDF/WAO guideline, which combines key symptoms, i.e. wheal numbers and pruritus intensity over seven consecutive days (UAS7). A modified version (modUAS7) with assessment of symptoms twice daily was used in some studies.

Objective: To better characterize both UAS7 versions with regard to their internal consistency, sensitivity to change and minimal important difference (MID).

Methods: 130 adult patients with antihistamine-refractory CSU completed both UAS7 versions, a Patients Global Assessment of disease activity (PatGA), the Urticaria Control Test (UCT), Chronic Urticaria Quality of Life Questionnaire (CU-Q2OL) and Dermatology Life Quality Index (DLQI) before and after initiation of therapy with omalizumab.

Results: Convergent validity: The UAS7 and modUAS7 showed high correlation with anchors for disease activity (PatGA): r=.568 (P<.001) and r=.605 (P<.001), respectively, and control (UCT): r=−.580 (P<.001) and r=−.585 (P<.001). Internal consistency: The wheal and pruritus scores of the UAS7 and modUAS7 correlated well with each other and showed an acceptable internal consistency (r=.64 (P<.001), Cronbach's α=0.78, and r=.63 (P<.001), Cronbach's α=0.77). Sensitivity to change: Changes in the UAS7 and modUAS7 correlated well with changes in the UCT score (r=−.642 (P<.001) and r=−.703 (P<.001)) and PatGA (r=639 (P<.001) and r=.763 (P<.001)). MID: The MID calculated using receiver operating characteristic (ROC) curve analysis and PatGA as anchor was found to be 11 for the UAS7 and of 12 for the modUAS7.

Conclusion: Both, the UAS7 and modUAS7 show good clinimetric properties, including a good sensitivity to change with MIDs of 11 and 12, respectively.

P098 | Incidence rates of sexually transmitted infections in "high-risk" men who have sex with men – a meta-analysis of trials and cohort studies on HIV pre-exposure prophylaxis

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Background: With the introduction of pre-exposure prophylaxis for the prevention of HIV transmission (HIV-PrEP) in the US and its approval in Europe, a new effective form of primary prevention for men who have sex with men and who engage in high-risk sex practices ("high-risk MSM") has become available. At the same time, epidemiological data depict increasing incidence rates of other sexually transmitted infections (STI). Data on the incidence of STI in high-risk MSM are scarce. This systematic review aims at analyzing data on the incidence of STI available from published HIV-PrEP studies.

Methods: MEDLINE, EMBASE and Cochrane CENTRAL were searched for clinical studies of PrEP in high-risk MSM that reported data on the incidence of STI during the follow-up. Incidence rates (events/100 person-years, py) with 95% confidence intervals (95%-CI) were calculated from the available data. PY of follow-up were determined from the number of participants per visit. In order to critically appraise the validity of the data, included studies were evaluated for three quality criteria: 1.) application of proper methods for the detection of STI, 2.) sufficient study size [i.e. ≥500py of follow-up], and 3.) sufficient follow-up (i.e. <20% discontinuation).

Results: The literature search yielded 1294 records; 135 of those were included during the title and abstract screening. Nine publications on seven studies (three double-blind RCTs, one open-label RCT, two cohort studies and one retrospective analysis of insurance data) met the inclusion criteria and reported data on the incidence of STI.

Seven studies reported data on the incidence of syphilis: four RCTs (9.28/100py, 95%-CI: 7.01-12.29), two cohort studies (9.23/100py, 95%-CI: 5.59-15.22) and one retrospective analysis of insurance data (9.31/100py, 95%-CI: 6.72-12.90). The overall estimate was 9.18/100py (95%-CI: 7.63-11.05). When only considering those studies that fulfilled at least two of the quality criteria (three RCTs, one cohort study), the overall estimate was 9.57/100py (95%-CI: 7.29-12.58).

For gonorrhea of all localisations, incidence rates could be calculated from two RCTs (29.15/100py, 95%-CI: 14.61-58.17), one cohort study (43.00/100py, 95%- CI: 37.52-49.28) and one analysis of insurance data (47.41/100py, 95%-CI: 41.03-54.78).

Data on chlamydia infection of all localisations were similar: two RCTs (23.14/100py, 95%-CI: 15.55-34.42), one cohort study (48.00/100py, 95%-CI: 42.19-54.61) and one analysis of insurance data (55.88/100py, 95%-CI: 48.91-63.83).

For the combined outcome of rectal gonorrhea and/or chlamydia infection, data were extracted from one RCT (36.56/100py, 95%-CI: 31.46-42.49) and one analysis of insurance data (55.88/100py, 95%-CI: 48.91-63.83).

Regarding hepatitis C infection, an overall incidence rate of 1.12/100py (95%-CI: 0.65-1.92) was calculated from data derived from two RCTs (1.23/100py, 95%-CI: 0.68-2.22) and one analysis of insurance data (0.66/100py, 95%-CI: 0.16-2.63).

Discussion/Conclusions: Despite the heterogeneous designs of the included studies and partly heterogeneous results, the data from all included studies depict high incidence rates of different STI among high-risk MSM. The high rates of STI in the analysis of insurance data of HIV-PrEP users, reflecting a "real-world" setting, particularly raise concerns. However, it is important to bear in mind that the presented data were derived from studies that were not designed to generate data on STI incidences. The data reflect estimates of STI acquisition in the group of "high-risk MSM" and are not directly associated with the intake of HIV-PrEP.

P099 | Eleven years of melanoma patient management – observations and trends from a single-center study in Austria

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Malignant melanoma (MM) accounts for 90% of all skin cancer related deaths. Recent studies indicate a constant increase of incidence rates over the last decades. Mortality rates, however, remained largely unchanged, which is thought to be the result of improved early detection and treatment, better management strategies, and rising public awareness for the importance of regular skin examinations. The aim of this study was to characterize the cohort of melanoma patients managed at a single-center institution in Vienna between 2000 and 2010. Parameters analyzed included sex, age at diagnosis, tumor stage, localization of the tumor, histological tumor type and sentinel lymph node (SLN) involvement among others. 1329 patients with a mean age of 59.116.7 years at diagnosis were analyzed. We found equal gender distribution (♀ n=669/50.3%, ♂ n=660/49.7%). Women were significantly younger than men at the time of melanoma diagnosis (♀ 57.217.8 years vs ♂ 61.0 15.2 years; P<.001). In contrast to data from the Statistic Austria database, which aims to monitor melanoma incidence rates for all of Austria, only a small number of patients were younger than 31 years (5.6% vs 36.2%). Most melanomas (83%) were diagnosed on typically sun exposed skin areas. Superficial spreading melanoma (39.5%) was the most frequent histological subtype, followed by nodular melanoma (14.9%), lentigo maligna melanoma (5.2%) and acral melanoma (2.6%). In 25.8% of patients the histological subtype could not be determined. The mean Breslow thickness (BT) was calculated with 1.81 mm and consistently increased with the age of the patients (age group 31-40: 1.211.42 mm; age group 71-80: 2.322.63 mm). No differences in BT for different locations of the primary tumor were found. The vast majority of tumors were detected at tumor stages

IA/B (TMIS: 7.4%; T1a/b: 41.5%). None of the TMIS and 3.1% of patients with tumor stage IA progressed. Out of all SLN biopsies, 17.3% of patients had a positive SLN; of those, 38.3% progressed. Interestingly, 12.9% of sentinel negative patients also had disease progression. In total, 11.3% of all patients experienced progressive disease of which 70.7% succumbed to melanoma. The number of patients with progressive disease increased form 3.1% when diagnosed at clinical stage IA to 50.0% for clinical stage IIIC. In line with previous studies, women had a better 5-year overall survival compared to men (75.8% vs 63.6%; *P*=.025).

The findings of this study are, for the most part, in line with previous reports and highlight that early detection is effective for preventing metastatic spread. Yet, we did not observe a decrease of median BT at diagnosis during the study period of 11 years. This might be explained by the high number of clinical stage IB patients, which require hospitalization for SLN biopsy. Alternatively, this could also indicate that melanoma awareness campaigns of the recent past need to be refocused.

P100 | Non-melanoma skin cancer awareness and protective behavior in outdoor professions

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Background: Non-melanoma skin cancer (NMSC) is the most common cancer worldwide. Outdoor workers are highly exposed to UV radiation and have a high risk for NMSC. Yet, evidence-based prevention programs for outdoor workers are not yet available but highly needed.

Objective: To assess UV protective behavior and NMSC awareness in different outdoor professions as a prerequisite for the development of prevention campaigns.

Methods: Cross-sectional study with a self-descriptive online survey among different occupational groups (farmer, gardener, roofer) in Germany. Logistic regressions were calculated to determine factors associated with different sun protection behaviors.

Results: Between February and April 2016 353 outdoor workers participated in the study. Of these, 67.4% reported, that they had never undergone a skin cancer screening by any medical doctor. Furthermore, 31.4% reported, that they had never heard of a skin cancer screening and 43.4% never use sun screen during their outdoor work. Inadequate use of sunscreen was more likely in male study participants (OR, 2.51; 95% CI, 1.26-5.24) and farmers (OR, 2.31; 95% CI, 1.14-4.85). A low perceived skin cancer risk was significantly associated with inadequate use of sunscreen (OR, 3.16; 95% CI, 1.75-5.84).

Conclusions: Sun protection behavior of outdoor workers can be improved. Awareness campaigns for high risk groups could increase the perceived risk of skin cancer and enhance the knowledge of sun safety measures to lower the burden of disease of NMSC in outdoor workers.

P101 | Psoriasis and addictive behavior: an underestimated problem

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Psoriasis affects up to 4% of the general population with an enormous socioeconomical impact. Within the last few years substantial achievements have been made in understanding the pathogenesis of psoriasis, which led to the approval of a number of highly effective drugs. However, only a proportion of psoriasis patients actually receive best medical treatment. To investigate the association of psoriasis and addictions and its possible negative impact on treatment compliance, we screened psoriasis patients for the most common addictions in Germany. 102 patients with psoriasis treated at the University Department of Dermatology at Technical University of Munich were included between October 2015 and February 2016 and asked to fill out a paper-based self-reported anonymous questionnaire with 92 questions of validated screening tests for addiction (alcohol, nicotine, drugs and illegal drugs, gambling, food). The results were then compared to the federal report on prevalence of addictions in Germany in 2015. Of 102 patients, 57 showed addictive behaviour measured with the used screening tools. Thereof, 41% were regular smokers, 24% high risk drinkers, 11% at risk for drug abuse, 4% at risk for food dependency and 19% compulsive gamblers. Compared to the general population addictions were significantly higher for alcohol abuse (***P***<.005), nicotine (***P***<.00005) and gambling (***P***<.0001). Screening measures for addictions have to be promoted for the assessment of psoriasis and can be recommended for all doctors treating patients with psoriasis. Addictions negatively affect treatment compliance and might contribute to the undertreatment of patients with psoriasis in general. Parallel to new drug approvals and even more detailed insights into pathomechanisms of psoriasis, public health strategies and interdisciplinary approaches are essential for a general sustained psoriasis treatment as requested by patients and the WHO in their recent psoriasis resolution.

GENETICS

P102 | Functional characterization of XPG and its spontaneous splice variants during nucleotide excision repair

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The nucleotide excision repair (NER) pathway eliminates UV-induced (bulky) DNA lesions in the human genome. This is the main protection mechanism against malignant cellular transformation of skin cells as

demonstrated by the recessively inherited NER-defective disorder xeroderma pigmentosum (XP). In this work, we focus on the incision step of NER, which is essential for the error-free removal of bulky DNA adducts from the human genome. Dual incision and gap filling are highly connected and strongly regulated by the presence of XPG in a "cut-patch-cut-patch" mechanism. The presence of XPG stimulates the initial cleavage by XPF/ERCC1 as well as first patch DNA synthesis. The following cut and second patch synthesis are dependent on endonuclease activity of XPG in vitro. This prompted us to investigate the importance of the diverse functional domains of XPG, known for interactions with PCNA and ubiquitin, or endonuclease function, with regard to accurate NER and DNA repair synthesis via host cell reactivation (HCR) and unscheduled DNA synthesis (UDS), respectively. We have created several recombinant XPG mutants and studied the effects of their overexpression in XPG deficient primary fibroblasts. Additionally, the ability of physiologically occurring spontaneous XPG splice variants to complement XPG deficient cells was investigated.
Our data demonstrate that (1) the interactions of XPG with PCNA and ubiquitin are essential for accurate NER, (2) the endonuclease activity of XPG is partially dispensable for accurate NER, and (3) C-terminally truncated splice variants of XPG are able to catalyze accurate NER on a low level. An XPG endonuclease back-up mechanism was deciphered involving DNA2 and the endonuclease activity of Fen1. Furthermore, we propose the blockage of translesion polymerases during NER as a new function of XPG, as PCNA- and ubiquitin- interaction-defective XPG mutants resulted in immediate, but inaccurate DNA repair synthesis.

P103 | Functional relevance of spontaneous alternative splice variants of the xeroderma pigmentosum group F gene

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The nucleotide excision repair (NER) pathway is a central DNA repair mechanism to repair a variety of bulky DNA lesions. Accumulation of DNA damage results in a cancer prone cellular mutator phenotype as demonstrated in patients with the autosomal recessive disease xeroderma pigmentosum (XP). A decreased NER capacity as a risk factor for several cancer entities in the normal population is well established. Components of the NER pathway already serve as risk biomarkers for cancers (e.g. XPG in melanoma) and their treatment outcome. The endonucleases XPF/ERCC1 and XPG are the core components of the incision complex of the NER and the heterodimer XPF/ERCC1 is also involved in repair of DNA interstrand crosslinks (ICLs).
We generated an XPF CRISPR/Cas9 knockout in MRC5Vi cells to analyze the unclear function of physiological spontaneous XPF mRNA splice variants. XPF knockout cells are viable, devoid of any XPF protein expression, and highly sensitive to NER and ICL repair substrates like UVC (LD50<1J/m2), cisplatin (LD50=0.125 μg/mL) and trimethylpsoralen in combination with UVA irradiation (LD50=0.5 ng/mL) in comparison to the wild-type cells (LD50=50J/m^2, LD50=1.5 μg/mL, LD50=13.5 ng/mL). We could not detect ERCC1 protein expression in the nucleus of the knockout cells, whereas there was a stable protein expression in the cytosol, implicating the necessity of a functional ERCC1/XPF heterodimer to allow ERCC1 to enter the nucleus. In our functional analyses of repair capabilities using a reporter gene assay, we identified two XPF splice variants (XPF-201 and XPF-003) with residual NER repair as well as ICL repair capabilities. XPF-201 only lacks the first 12 amino acids of the protein, while XPF-003 is severely C-terminally truncated. Interestingly, in contrast to XPF-202 which differs to XPF-003 only in the first 12 amino acids, XPF-202 splice variant has no repair capability, suggesting an importance of these first 12 amino acids in interacting with other proteins involved in the repair pathways. We suppose that this part of the protein is important for interaction with SLX4 and are now further investigating this. Additionally, we focus on the involvement of XPF/ERCC1 in the repair of double strand breaks. Finally, the discovery of physiologically occurring splice variants with residual repair capability may be used for the development of prognostic markers for individual repair capability and therefore disease outcome and therapy success.

P104 | Tracing of SNPs within Psoriasiform Skin Disease-associated locus 1 (PSD1) via Next generation Sequencing

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Psoriasis is an autoimmune disease of skin typically characterized by itchy red skin with silvery scales and affects 2%-3% of the general population. Previously using a congenic approach, we identified a 9-cM fragment on chromosome 10 causing psoriasiform disease in CD18hypo PL/J mouse model and designated this locus as psoriasiform skin disease associated locus 1 (PSD1). In order to get more detailed insight regarding the involvement of genes present in PSD1 locus in the pathogenesis of the psoriasiform phenotype. We have analyzed SNPs underlying PSD1 locus in susceptible CD18hypo PL/J mice and resistant CD18hypo C57BL/6J mice using next generation sequencing. We performed the local realignment and analysis using the C57BL/6J mouse reference genome (NCBIM37/mm9) with BWA software and Genome Analysis Toolkit (GATK). The Annotations and discovery of SNPs and indel was performed using ANNOVAR, SAM and BCF tools. We analysed all exons, flanking regions and introns of the PSD1 locus on chromosome 10 for possible SNPs, excluding CD18-based differences, heterozygous and intergenic SNPs and

identified 155 homozygous SNPs in PSD1 locus. Interestingly, out of these 155 homozygous SNPs, 142 of these SNPs are already known, while the remaining 13 SNPs have not been described. To explore the possible role of these 13 SNPs is psoriasiform disease observed in CD18 hypo mice, further validation and a detailed expression analysis is needed.

P105 | Survival of induced pluripotent stem cells clearly depends on PI3K/AKT signaling pathway

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Induced pluripotent stem cells (iPSCs) are a type of pluripotent stem cells and can be artificially generated from all somatic cell types of individual organisms. They are largely similar to embryonic stem cells in the essential properties of morphology and pluripotency. Human iPSCs are of great hope for regenerative medicine due to their broad potential to differentiate into specialized cell types in culture. They may be useful in research on development of tissues, drug screening or disease mechanisms and may provide the basis for future cell-based replacement therapies. However, there is only poor insight into iPSC signaling, e.g. regulation of apoptosis. Apoptosis is known as programmed cell death. It contributes to maintaining tissue homeostasis and normally eliminates highly proliferative cells with malignant properties. The aim of our study has been to investigate the effects of five biologically relevant kinase inhibitors (e.g. Aurora kinase inhibitor) as well as of the death ligand TRAIL on apoptotic response of fibroblast-derived iPSCs obtained from M. Alzheimer patients and healthy controls. Interestingly, we found that the high basal apoptotic rate of iPSCs is strongly suppressed by the pan-caspase inhibitor QVD-Oph, thus underlining the dependency on proapoptotic caspase cascades. Furthermore, wortmannin, an inhibitor of phosphoinositid-3 kinase/Akt signaling (PI3K-AKT), dramatically and rapidly induced apoptosis in iPSCs. In contrast, parental fibroblasts as well as iPSC-derived neuronal cells were not responsive to inhibitors used here. The resulting condensation and fragmentation of DNA and decrease of the membrane potential in iPSCs are typical features of apoptosis. Comparable effects were observed with an AKT inhibitor (MK-2206). Wortmannin resulted in disappearance of phosphorylated AKT and activation of the main effector caspase-3 in iPSCs. Our results demonstrate for the first time that PI3K-AKT represents a highly essential survival signaling pathway in iPSCs. These findings give further insight in the underlying mechanisms of apoptosis regulation in iPSCs.

P106 | Global RNA expression profiling in skin provides insights into disease mechanisms of atopic eczema and psoriasis

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Previous studies on RNA expression in skin have indicated the presence of distinct gene expression signatures in patients with inflammatory skin diseases such as atopic dermatitis and psoriasis. However, many of these studies suffer from small sample sizes, imperfect patient matching, and limited data availability due to application of microarrays. We here assessed the cutaneous transcriptome in lesional, non-lesional and healthy skin of 32 AD patients, 36 Psoriasis patients and 43 healthy controls, respectively, carefully matched for age, sex, and site of biopsy, using next generation sequencing.

Differential gene expression was analyzed with DESeq2 and functional annotation was performed using the R-package goseq and the Gene Ontology database. DEGs were defined by absolute log2-fold change >1 and a false discovery rate <0.05.

In total 15.216 unique genes out of >29.000 analyzed genes were expressed across all skin types. First analyses identified 2.146 differentially expressed genes (DEGs) in lesional AD (AL) vs healthy skin (NN) and 4.878 DEGs in lesional psoriatic (PL) vs NN skin. 1283 DEGs in AL skin and 1861 transcripts in PL skin were increased, whereas 863 DEGs in AL skin and 3.017 transcripts in PL skin showed a decrease. Comparison of non-lesional patient and healthy control skin revealed 7 genes to be upregulated in AD (AN), and 9 genes to be upregulated in Pso (PN) patients. The majority of DEGs in lesional and non-lesional skin identified in both diseases are involved in different inflammatory immune processes and epidermal differentiation. Pathway analysis revealed a significant overrepresentation of genes involved in e.g. TH1, TH2 and TH17 cell differentiation and activation, epidermal proliferation and antimicrobial defense.

Further analyses are currently performed to comprehensively define underlying molecular pathways and to compare and contrast RNA expression differences between the two inflammatory skin diseases AD and Pso.

P107 (OP05/04) | Downregulation of keratin 79 upregulates LRIG1 and induces sebaceous gland hyperplasia

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In adult murine skin, keratin 79 (KRT79) is exclusively expressed in the cells of the hair follicle infundibulum (INF), in mature sebocytes, and in duct cells of the sweat glands. So far, gene targeting studies of the INF had to employ driver mouse lines using a KRT5 or KRT14 promoter, which are also widely active in the hair follicle and in the basal layer of the interfollicular epidermis (IFE). To target genes in the INF more specifically, we established a mouse line with KRT79-specific expression of a tetracycline transactivator (tTA). We replaced the first exon of the Krt79 gene with the cDNA for tTA via homologous recombination in embryonic stem cells. After obtaining germline transmission of the modified allele via chimeric mice, we crossed the positive offspring to a HA tagged mouse line to assess the specificity of the expression. To our surprise, the heterozygous Krt79wt/tTA animals showed a striking skin phenotype: Krt79wt/tTA mice developed wrinkled skin, skin inflammation, and a greasy hair coat. This phenotype increased dramatically in homozygous Krt79wt/tTA mice.

Histological analysis revealed a strongly increased epidermal thickness and enlarged sebaceous glands in Krt79wt/tTA mice compared to wild-type littermates. The INF of Krt79wt/tTA mice showed significantly more Ki67 positive cells compared to control littermates, and a Western blot for PCNA revealed an increased proliferation in the skin of Krt79wt/tTA mice. We also observed accumulation of mast cells in the dermis of Krt79wt/tTA mice. Measurements with a Sebumeter indicate that the hyperplasia of the sebaceous glands is connected with an increased sebum production in Krt79wt/ tTA mice.

We observed that hetero- and homozygous mice were often scratching themselves and developed skin wounds on these areas. Blood analysis revealed increased white blood cells, neutrophil cells, and basophil cells in Krt79wt/tTA mice. At necropsy the skin weight of Krt79wt/tTA animals was increased and we additionally observed enlarged spleen and lymph nodes.

It is known that the pool of LRIG1-positive stem cells in the hair follicle isthmus gives rise to KRT79 positive cells in the INF and the sebaceous gland, which then lose LRIG1 expression with ongoing differentiation. At postnatal day 3 KRT79 expressing cells are still located in the beginning of the IFE and have a weak expression of KRT10; in adult mice KRT79 is not expressed in the IFE and KRT79 positive cells lost KRT10 expression. In control animals LRIG1-expression is limited to the isthmus and KRT10 positive cells are located in the supra basal layer of the IFE. KRT79 separates in control mice KRT10 positive cells from the LRIG1 stem cells in the isthmus. In Krt79wt/tTA mice, however, we detected LRIG1 positive cells and KRT10 expressing cells along the whole INF and in the IFE.

Our results suggest that KRT79 is important to keep LRIG1 stem cells in the isthmus and restrain the stem cells from migrating into the INF and IFE. On the other hand KRT79 seems to keep KRT10 positive cells in the IFE and restrain them from migrating into the hair follicle. In the absence of KRT79, LRIG1 positive cells in the INF and IFE may proliferate abnormally and induce pathological changes. Interestingly, it has been shown that comedones in human acne are free of KRT79 and express KRT10; these cysts are filled with keratin debris, which were also often observed in Krt79-/tTA mice.

P108 | Whole-exome sequencing of a xeroderma pigmentosum patient reveals extremely high mutational loads in basal cell carcinomas

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Xeroderma pigmentosum (XP) is a rare autosomal-recessive disorder caused by a defect in post-UV DNA repair which results in a high incidence of skin cancers on sun-exposed skin areas. In contrast to sporadic forms of skin cancer, the mutational landscape of XP tumours has not been comprehensively characterised, yet.

We applied whole-exome sequencing to two basal cell carcinomas (BCC), two Bowen's disease lesions, one actinic keratoses and a non-lesional skin biopsy from the upper extremities, as well as blood of a 51 years old patient suffering from the XP E-type showing multiple non-melanoma skin cancers (NMSC) and metastatic melanoma. Illumina TruSeq Exome library was used for exome capture and sequenced on Illumina HiSeq 3000, generating 2 × 75 bp paired-end reads and an average target coverage of 73×. Variant calling was performed using Mutect v.1.1.4 and VarScan v2.4.0 software. Mutect was used to call somatic SNVs and VarScan was used to call somatic loss-of-heterozygosity (LOH) mutations and somatic indels.

Healthy skin, M. Bowen 1 & 2, actinic keratoses and basal cell carcinomas 1 & 2 showed somatic SNV rates of 2, 1, 3, 10, 280 and 342 mutations/Mb, respectively. The mutational burden of the XP-BCCs (11530 and 14373 mutations) was considerably higher than that described for sporadic forms of BCCs, with, however, limited overlap of 108 somatic SNVs only. None of those SNVs overlapping both XP-BCCs was observed in 100 sporadic BCCs. The mutation rates of the Bowen lesions were smaller than that of the actinic keratoses. The prevalence of somatic SNVs varied much stronger among the skin samples than that of somatic indels and somatic LOH indels pointing to the major role of SNVs in skin cancer development. The five lesional sites shared 14 somatic SNVs, of which 2 located in the NBPF9 and C22orf43 were not detected in non-lesional skin and could represent early-stage mutations. In this XP patient, the skin tumours are characterised by a massively increased mutational burden, but potentially diverse molecular mechanisms suggesting that XP patients may benefit from (early) treatment with PD-1 inhibitors. The therapy response of melanoma patients to these biological correlates with mutational load of the tumours, as well as with the presence of BRCA2 mutations. Interestingly, both BCCs of the XP patient carry a nonsynonymous mutation in BRCA2, of which one is associated with cancer development.

P109 | Phenotype diversity in autosomal recessive congenital ichthyosis associated with mutations in the ST14 gene

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Autosomal recessive congenital ichthyosis (ARCI) is a heterogeneous group of non-syndromic keratinization disorders. Currently, at least 19 different types can be distinguished. ARCI type 11 (OMIM 602400), also known as autosomal recessive ichthyosis with hypotrichosis (ARIH), is caused by homozygous mutations in the ST14 gene. Clinically, the disorder is characterized by congenital ichthyosis with curly, sparse hair with or without follicular atrophoderma, and/or hypohidrosis. We studied a 45-year-old Caucasian male of Russian origin who suffered from generalized ichthyosiform erythroderma with severe itch since birth. Of note, there were no further cutaneous or extracutaneous symptoms, in particular no hair or other ectodermal abnormalities, neither at birth, nor during childhood or adulthood. Further, there was no family history of any skin disorder. Using gene panel sequencing for ARCI, we identified a homozygous splice-site mutation in intron 5 of the ST14 gene, c.598+1G>A (IVS5+1G>A). Interestingly, the same mutation has been previously reported in a 4-year-old girl with ARCI from a consanguineous Kuwaiti family who also had diffuse hypotrichosis. Even more interestingly, all other patients with ARCI caused by ST14 mutations reported to date likewise showed hypotrichosis. Thus, the patient presented here is the first one indicating marked phenotype diversity in ARCI associated with ST14 mutations. Based on our findings we suggest that the nomenclature of this subtype of ARCI should be revised because the hitherto assumed genotype-phenotype correlation apparently does not hold true.

P110 | Late-onset erythropoietic protoporphyria caused by mosaicism after autologous blood stem cell transplantation and radiation therapy

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Erythropoietic protoporphyria (EPP) is an autosomal semi-dominant disorder that is due to a marked deficiency of ferrochelatase (FECH), the eighth enzyme in heme biosynthesis. This enzymatic dysfunction results from inheritance of a germline FECH gene mutation on one parental allele in combination with a common hypomorphic intronic FECH variation, IVS3-48C, on the other parental allele. Usually, EPP manifests in early childhood with burning cutaneous photosensitivity. Here we present a 57-year-old Caucasian man who developed severe cutaneous photosensitivity six years after treatment of a large B-cell lymphoma with autologous blood stem cell transplantation and radiation therapy. Biochemical and enzymatic analyses confirmed the diagnosis of EPP. By leukocyte DNA sequencing we detected a nonsense mutation, p.R298X, in combination with the hypomorphic IVS3-48C variation in trans. Interestingly, the chromatographic intensity of the mutated T-allele at position 298 was reproducibly lower than that of the wild-type C allele, suggesting mosaicism. Most of the rare cases of late-onset EPP occurred in association with a myelodysplastic syndrome or myeloproliferative disorder due to a deletion on chromosome 18q, the region in which the FECH gene is located. Hence, we first excluded such a deletion by fluorescence in situ hybridization. Sequencing of DNA derived from cultured fibroblasts of the patient showed absence of p.R298X, confirming that this mutation arose as a result of mosaicism. This is the first report on late-onset EPP due to blood cell mosaicism caused by a spontaneous FECH mutation following autologous blood stem cell transplantation and radiation therapy of a large B-cell lymphoma.

P111 | Meta-analysis of gene expression profiling of CD4+ T cells reveals novel shared mechanisms and markers between Pemphigus and Systemic lupus erythematosus

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Pemphigus diseases is a group of rare autoimmune diseases of skin and mucous membranes, mediated by autoantibodies against desmosomal adhesion molecules. Binding of the pathogenic autoantibodies to the target proteins leads to dissociation of adjacent keratinocytes and formation of blisters. The association of pemphigus with connective tissue diseases such as systemic lupus erythematosus (SLE) has been previously documented. However, the molecular mechanisms explaining this finding are still unclear. The co-occurrence of pemphigus and SLE could involve common network of multi-functional genes and pathways. Alternatively, it could be altogether stochastic. Regarding complexity of such system, we used weighted gene co-expression network analysis (WGCNA) as a comprehensive tool for identifying modules of correlating and connected shared genes. This approach has been previously successfully applied in various biological contexts to identify clusters (modules) of highly correlated genes and networks associated with the disease.

Even though systemic lupus erythematosus (SLE) and pemphigus were traditionally classified as B-cell-mediated diseases, compelling evidence has however shown that T lymphocytes are crucial in pathogenesis of both diseases by regulating B cells response and promoting autoantibody production. Using publically available microarray data and WCGNA, we investigated gene co-expression networks of CD4+ T-cells obtained from pemphigus and SLE patients. Our analysis reveals 15 distinct modules containing 3280 co-expressed genes between the two diseases, with two modules out of 15 significantly up-regulated in both pemphigus and SLE, or pemphigus alone. Consequent gene ontology analyses further revealed enrichment of type I interferon signaling and response to viral infection, as well as blood coagulation and platelet activation in these modules. During further investigation, we could identify several candidate hub genes, such as BCL2, STAT1,

and GBP1. By inclusion into analysis of previously reported GWAS data, additional distinct interactions of gene modules, revealed by our analysis, with already reported genes could be elucidated.

To the best of our knowledge, this is the first study applying systems biology approach to identify shared molecular mechanisms between pemphigus and SLE diseases. This method could broaden our knowledge about pathogenesis of autoimmune diseases by identifying new possibly involved candidate genes, as well as improve our understanding of underlying genetic interactions and reveal new potential therapeutic targets.

P112 | Diet shifts the genetic association of multiple complex traits in outbred mice

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Genome-wide association and mapping studies identified a multitude of genetic variants associated with complex traits in humans and mice, thus conveying detailed insights into their genetic architecture. Yet, these genetic variations only partially account for the phenotypic variability. This missing heritability may be due to epistasis, rare variations and/or the environment. We here addressed the later, by exposing a large colony of outbred mice to different diets. Mice were fed control chow or western diet ad libidum, or were held at caloric restriction (n=350-400 mice per group). We show that complex phenotypes depend on both, genetic architecture and diet. Full-genome sequencing of parental mice and forward genomics allowed linking the associations to single genes. Considering diet as an interactive variable to determine the gene-phenotype association, leads to a considerable shift of the genetic association. Thus, gene-diet interactions explain a significant part of the missing heritability, which allows a more detailed understanding of complex traits.

P113 (OP06/04) | Mutations in three genes encoding proteins involved in hair shaft formation cause uncombable hair syndrome

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Uncombable hair syndrome (UHS), also known as "spun glass hair syndrome," "pili trianguli et canaliculi," or "cheveux incoiffables" is a rare anomaly of the hair shaft which occurs in children and improves with age. UHS is characterized by dry, frizzy, spangly and often fair hair that is resistant to being combed flat. Up to date both simplex and familial UHS cases with autosomal dominant as well as recessive inheritance have been reported. However, none of these cases were linked to a molecular genetic cause. Here, we report the identification of UHS causative mutations located in the three genes PADI3 (peptidylarginine deiminase 3), TGM3 (transglutaminase 3) and TCHH (trichohyalin) in a total of eleven children. All of these individuals carry homozygous or compound heterozygous mutations in one of these three genes, indicating an autosomal recessive inheritance pattern in the majority of UHS cases. The two enzymes PADI3 and TGM3, responsible for posttranslational protein modifications, and their target structural protein TCHH, are all involved in hair shaft formation. Elucidation of the molecular outcomes of the disease causing mutations by cell culture experiments and tridimensional protein models demonstrated clear differences in the structural organization and activity of mutant and wild-type proteins. Scanning electron microscopy observations revealed morphological alterations in hair coat of Padi3 knockout mice. All together, these findings elucidate the molecular genetic causes of UHS and shed light on its pathophysiology, and hair physiology in general.

HEALTH SERVICES RESEARCH

P114 | Urticaria Activity Score – Results of the available versions are comparable

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Background: The signs and symptoms of chronic spontaneous urticaria (CSU) strongly fluctuate from day to day and a biomarker for disease activity is still missing. Currently, the only widely accepted tool to determine disease activity in CSU is the patient-reported Urticaria Activity Score (UAS). The UAS daily documents wheal numbers and intensity of pruritus, usually over 7 consecutive days (UAS7). While

the EAACI/GA²LEN/EDF/WAO guideline recommends one version of the UAS7, another modified version (modUAS7) has been validated and used in recent phase III studies. Both UAS versions differ in their frequency of documentation (once daily vs twice daily) and in their categories for wheal numbers. Objective

To assess the comparability of results obtained by the two available UAS versions.

Methods: 130 adult patients (73% female) with antihistamine-refractory CSU documented their disease activity with both UAS versions while waiting for treatment adjustment with omalizumab. Results

The mean UAS7 values SD (24.1 10.3) were slightly, but significantly lower as compared to mean modUAS7 values SD (25.7 10.7, $P<.001$). Expectedly, the wheal scores of the UAS7 (11.4 6.0) were slightly, but significantly lower as compared to those of the modUAS7 (13.6 6.4, $P<.001$), while the pruritus scores of the UAS7 (12.7 5.4) were slightly, but significantly higher as compared to those of the modUAS7 (12.1 5.5, $P<.01$). The results of the UAS7 and the modUAS7 strongly correlated with each other ($r=.90$, $P<.001$). Conclusion

The results of both UAS7 versions are largely comparable. However, it has to be taken into account that the modUAS7 scores are consistently slightly higher as compared to UAS7 scores. As compared to the modUAS7, the UAS7 is easier to handle (only once daily documentation) and easier to score, which supports its use in routine patient management.

P115 | Urticaria Activity Score – Scores representing mild, moderate and severe disease

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Background: The Urticaria Activity Score (UAS) is the current gold standard to assess disease activity in chronic spontaneous urticaria (CSU). It daily documents wheal numbers and intensity of pruritus, usually over 7 consecutive days (UAS7). While the EAACI/GA²LEN/EDF/WAO guideline recommends one version of the UAS7, another modified version (modUAS7) has been validated and used in recent phase III studies. Although the UAS is widely applied, data on the interpretability of its results are scarce.

Objective: To assess and compare which UAS7 and modUAS7 values are indicative of mild, moderate and severe CSU activity.

Methods: 130 adult patients (73% female) with antihistamine-refractory CSU documented their disease activity with both UAS versions while waiting for treatment adjustment with omalizumab. In addition, all patients were asked to globally self-rate their urticaria activity as mild, moderate, or severe (PatGA).

Results: The mean UAS7 and modUAS7 values SD of patients who self-rated their disease activity to be mild, moderate, or severe were 15.4 7.1 and 16.6 8.1, 22.5 9.1 and 23.6 9.8, 31.2 8.5 and 33.7 6.6, respectively. The 25th and 75th percentile were 11 and 20 (UAS7) and 10 and 24 (modUAS7) for PatGA mild, 16 and 30 points (UAS7) and 16 and 32 points (modUAS7) for PatGA moderate, and 27 and 37 (UAS7) and 28 and 40 points (modUAS7) for PatGA severe. Expectedly, the differences between UAS7 and modUAS7 values were primarily driven by the wheal component. ROC curve analyses suggested cut-off value for moderate to severe disease of 17 (UAS7) and 20 (modUAS7).

Conclusion: Our data is important for the interpretation of UAS values obtained in clinical studies and routine management. As expected from the composition of the available UAS versions, the modUAS has a higher cut-off value for moderate to severe disease activity.

P116 | Urticaria Control Test – Responsiveness and minimal important difference

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Background: The Urticaria Control Test (UCT) is a globally used and universal patient-reported outcome measure for measuring disease control in chronic urticaria patients. As of yet, its responsiveness has not been established.

Objective: The aim of this study was to investigate the UCT's ability to detect changes over time, including the minimal important difference (MID) and the smallest detectable change (SDC). Methods

Sixty-five antihistamine-refractory CSU patients used the UCT to document their disease control as well as several anchor instruments for disease activity, disease control, health-related quality of life, and treatment response before and 4 weeks after the initiation of omalizumab therapy. The UCT's sensitivity to change was assessed by correlating its score changes with changes in the applied anchors. In addition, the MID and SDC were calculated by using distribution-criterion and anchor-based approaches.

Results: After the initiation of omalizumab, UCT scores markedly improved as compared to pretreatment levels. The UCT score changes correlated strongly with changes of disease activity and health-related quality of life. In addition, UCT results and their changes were well in accordance with the patient's assessment of their treatment efficacy, their disease control, and with the patient's response to treatment. The MID and SDC of the UCT were found to be 3 and 4 points, respectively.

Conclusion: The UCT score is sensitive to change. Accordingly, the UCT is a valuable tool to assess levels but also changes of disease control in patients with chronic urticaria over time, e.g. before and after treatment adjustment.

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P117 | IL-2 functionalized hydroxyethylstarch nanocapsules for targeting of human regulatory CD4$^+$CD25^{high} T cells in vitro and in vivo

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Due to an increased efficiency and a reduction of side effects, targeted drug delivery by use of cell-type specific nanoparticles is a promising approach for delivery of toxic or instable agents. In tumor immunotherapy, targeting of antigen presenting cells is a frequently used concept, exploiting their high endocytotic activity. In contrast, targeted drug delivery to T cells remains an obstacle. In the present study, we generated IL-2 functionalized hydroxyethylstarch nanocapsules (HES-D-IL-2 NC) to target CD25 (IL-2 receptor alpha chain) positive T cells. Flow cytometry and laser scanning microscopy experiments indicated an enhanced uptake of HES-DIL- 2 in comparison with dibenzylcyclooctyne (DBCO)-functionalized control (HED-D) capsules by human activated CD4$^+$CD25$^+$ T cells. The observed uptake was shown to be CD25$^+$ dependent as CD25$^+$ and CD25^{high} T cells revealed a significantly enhanced NC incorporation compared to CD25$^-$ T cells. Furthermore, CD25 blocking by an anti-human CD25 monoclonal antibody Simulect (basiliximab) significantly inhibited the uptake of HES-D-IL-2 but not of HES-D NC by human CD4$^+$CD25$^+$ T cells. To target T cells with different IL-2 receptor affinities we generated NC with a twofold (HES-D-IL-2$_{/2}$) or tenfold (HES-D-IL-2$_{/10}$) reduced amount of IL-2 on their surface. Comparative studies of human naïve CD25$^-$ vs activated CD25$^+$ vs regulatory CD25^{high} CD4$^+$ T cells revealed significantly differences in the HES-D-IL-2 NC uptake due to different IL-2 receptor affinities, resulting in a very low incorporation of HES-D-IL-2 NC in naïve and a moderate or high uptake by activated or regulatory T cells, respectively. In contrast, regulatory T cells incorporated HES-IL-2$_{/2}$ and HES-D-IL-2$_{/10}$ to the same extent as shown for HES-D-IL-2, indicating a high IL-2 sensitivity. In addition, in vivo studies using human T cell- or PBMC-reconstituted RAG2$^{-/-}\gamma c^{-/-}$ mice indicated a significantly enhanced uptake of the HES-DIL- 2 NC by CD4$^+$CD25$^+$ T cells compared to control NC.

In summary, we were able to generate IL-2 functionalized NC to target human CD4$^+$CD25$^+$ T cells with different IL-2 receptor affinities in vitro and in vivo.

P118 | Dysregulation of pro- and anti-inflammatory Th17 cells in autoinflammatory syndromes

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Background: Th17 cells are crucial mediators of autoimmune inflammation. However, two distinct types of Th17 cells have recently been described, which differed in their polarization requirements for IL-1β and thus in their cytokine repertoire, of note in their ability to produce IL-10. Whether these distinct Th17 phenotypes translate into distinct Th17 cell functions and whether this has implications for human health or disease has not been addressed yet.

Objective: We hypothesized that IL-1β-independent Th17 cells have anti-inflammatory functions whereas IL-1β-dependent Th17 cells are pro-inflammatory due to a dominant function of differentially expressed IL-10 and irrespective of other secreted molecules that determine the full cytokine profile of the Th17 cell subsets. Considering the crucial role of IL-1β in the pathogenesis of autoinflammatory syndromes, we hypothesized that IL-1β mediates the loss of anti-inflammatory Th17 cell functionalities in Schnitzler syndrome, an autoinflammatory disease.

Methods: To assess pro- vs anti-inflammatory Th17 cell functions we performed suppression assays and tested the effects of IL-1β-dependent and IL-1β-independent Th17 subsets on modulating pro-inflammatory cytokine secretion by monocytes. Schnitzler syndrome patients were analysed for changes in Th17 cell functions before and during therapy with IL-1β blocking drugs. The results were corroborated with patient samples from another autoinflammatory syndrome, systemic juvenile idiopathic arthritis.

Results: IL-10+ Th17 cells, which differentiated independently of IL-1β, have regulatory functions similar to Treg cells while IL-1β-dependent IL-10- Th17 cells have not. Both Th17 cell subsets differ in their ability to suppress T cell proliferation as well as in their ability to modulate pro-inflammatory cytokine production by antigen presenting cells. In Schnitzler syndrome systemic overproduction of IL-1β translates into a profound loss of anti-inflammatory Th17 cell functionalities, which can be reversed by anti-IL-1β treatment.

Conclusion: IL-1β signaling determines the differential expression pattern of IL-10, which is sufficient to confer immunosuppressive vs pro-inflammatory Th17 cell functionalities to Th17 cell subsets. Our data introduce Th17 cell subsets as novel players in autoinflammation and thus novel therapeutic targets in autoinflammatory syndromes including other IL-1β-mediated diseases. This demonstrates for the first time alterations in the adaptive immune system in autoinflammatory syndromes.

P119 | Immuno-modulatory effects of prebiotics, probiotics and active microbial structures on human primary keratinocytes and human primary nasal epithelial cells

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The human skin is one of the largest immunologic organs and represents the interface between the body and the environment. To achieve an effective defense barrier against environmental insults a crosstalk between epithelial and immune cells as well as the skin's microbiota has to be maintained. Besides direct cell-cell contact also indirect contact via the exchange of soluble mediators such as cytokines is used to transfer information. The release of these mediators is assumed to be potentially influenced by "beneficial intervention factors" such as prebiotics, probiotics or active microbial structures (e.g. non-digestible fiber compounds, lactic acid bacteria, bifidobacteria, microbial proteases). However, the underlying mode of action as well as a direct contribution to skin health is not clear to date. To gain deeper insight in this field we investigated whether pre-/probiotics or active microbial structures have a direct effect on immune regulation as well as barrier function of human keratinocytes and human nasal epithelial cells.

For this purpose human primary keratinocytes and nasal epithelial cells were stimulated with a specific mixture of non-digestible short-chain galactooligosaccharides (GOS) and long-chain fructo-oligosaccharides (FOS) alone or in a combination with either lactic acid bacteria or lactocepin, a PrtP-encoded cell envelope protease of lactococci. To simulate inflammatory conditions, cells were costimulated with IFN-γ/TNF-α. Furthermore, the prebiotics were tested regarding their effect on transepithelial electrical resistance (TEER) in an air-liquid interface model after stimulation with IL-4 and IL-13.

Results revealed that the presence of GOS/FOS decreases the secretion of pro-inflammatory cytokines such as IP-10 and CCL-5 while combining GOS/FOS with probiotic bacteria leads to an even more pronounced effect. The strongest effect, however, could be observed in the presence of lactocepin. In contrast, the release of galectin 9, a known supporter of regulatory T cell functions, was enhanced in response to GOS/FOS in human keratinocytes. Furthermore, it could be observed that GOS/FOS dampens the decrease of transepithelial electrical resistance after stimulation with IL-4 and IL-13.

Taken together the current study shows that pre- and probiotics as well as active microbial structures can indeed influence inflammatory processes and barrier function in human keratinocytes and nasal epithelial cells and can act as regulatory compounds. This supports our hypothesis that pre-/probiotics and active microbial structures can directly impact on human epithelial cells and might have the potential to be used therapeutically for the maintenance and repair of epithelial integrity in future.

P120 | Inhibition of phosphodiesterase 4 in 6-sulfo LacNAc (slan) dendritic cells enhances their capacity to induce Th17 responses

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Increasing intracellular levels of cyclic adenosine monophosphate (cAMP) by inhibition of phosphodiesterase 4 (PDE4) is a therapeutic strategy in a number of pro-inflammatory diseases. The PDE4 inhibitor apremilast is currently licensed for the treatment of psoriasis and psoriasis arthritis; however its immune modulatory properties are not well understood. In this study we describe the immune regulatory effects of PDE4 inhibition at the level of 6-sulfo LacNAc dendritic cells (slanDCs), a population of DCs that is known to produce high amounts of pro-inflammatory cytokines and to induce strong Th17/Th1 T cell responses. In line with published data on other cell types, treatment of slanDCs with apremilast reduced their production of IL-12 and TNF-α. Accordingly, T cells from psoriasis patients or healthy donors that were stimulated by apremilast-treated slanDCs yielded reduced amounts of the Th1 cytokine IFN-γ, and in parallel, expressed decreased levels of the transcription factor T-bet. On the contrary, we observed a strong stimulation of Th17 responses. slanDCs treated with apremilast expressed high levels of IL-23p19 mRNA and secreted increased amounts of IL-23 and IL-1β. T cells stimulated by these slanDCs revealed a strong IL-17 production and an upregulated expression of the transcription factor RORγt. Altogether, these results indicate an immune regulatory rather than a general immune suppressive function of PDE4 inhibitors at the level of human DCs which should be considered in the treatment of Th17-mediated diseases.

P121 | Invariant natural killer T (iNKT) cells are reduced in peripheral blood of bullous pemphigoid patients and enriched in lesional skin

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Invariant natural killer T cells (iNKT cells) are a subset of T lymphocytes characterized by expression of an invariant T cell receptor (iTCR) alpha chain (V alpha 24-J alpha 18) paired with a V beta 11 chain. iNKT cells may mediate both pathogenic inflammation and regulatory immune functions, and they have been shown to play a role in the pathogenesis of several chronic diseases, e.g. lupus erythematosus and atopic dermatitis. Bullous pemphigoid is the most frequent bullous autoimmune dermatosis associated with autoantibodies against hemidesmosomal proteins. In addition to autoreactive B cells activation of the innate immune system may contribute to the disease pathogenesis.

In this study, we investigated the frequency of iNKT cells in peripheral blood (PB) and skin biopsies from lesional and non-lesional skin from patients with bullous pemphigoid and controls.

PB was obtained from 30 patients with bullous pemphigoid (aged 59-94 years, mean 81.6) and from 30 controls (20 patients with skin tumors and 10 healthy controls, aged 24-84, mean 50.6). Duration of pruritus and skin lesions of bullous pemphigoid patients varied

between a few days and 6 months. The patients were included in the study upon primary diagnosis of bullous pemphigoid by histology, direct immunofluorescence and BP180/BP230 ELISA.

The frequency of CD3+/6B11+ iNKT cells was evaluated using flow cytometry. The number of V alpha 24+/V beta 11+ iNKT cells in 34 lesional and 14 non-lesional skin biopsies from patients with bullous pemphigoid and in healthy appearing skin from 17 patients with skin tumors was assessed by immunofluorescence staining.

Patients with bullous pemphigoid showed a significantly lower frequency of circulating CD3+/6B11+ iNKT cells in PB (median 0.023; IQR 0.009-0.037) compared to the control group (median 0.065; IQR 0.040-0.107, $P<.0001$).

The V alpha 24+/V beta 11+ iNKT cells were significantly enriched (10.5 cells/10 visual fields; IQR 7-16.5) in lesional skin of bullous pemphigoid patients compared to non-lesional skin (3 cells; IQR 1-6.5) or biopsies from control patients (2 cells IQR 1-4.5; $P<.0001$).

In summary, we demonstrate that iNKT cells are reduced in PB of bullous pemphigoid patients and enriched in lesional skin similarly to previous results in e.g. lupus erythematosus. We hypothesize that the reduced frequency of circulating iNKT cells in PB may be associated with migration of iNKT cells into the affected skin areas and that iNKT cells may play a pathogenic role in this common bullous autoimmune dermatosis of elderly individuals.

P122 | Activation of regulatory T cells by superagonistic antibodies to CD28 is determined by integrin αE (CD103)

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Regulatory T cells (Tregs) are indispensable for immune regulation. They are a subpopulation of CD4+ T cells characterized by CD25 and Forkhead transcription factor P3 (FoxP3) expression. So far, little is known about the regulation of Tregs themselves. The CD103+ Treg subset shows higher FoxP3 expression and enhanced suppressive capacities in vitro and in vivo compared to their CD103- counterparts. The connection between CD103, FoxP3 and Treg activation, however, remains largely enigmatic.

To induce Treg activation, we injected wild-type (WT) and CD103−/− mice with a superagonistic CD28 antibody (CD28SA), which causes antigen-independent expansion and activation of T cells, preferentially natural Tregs.

In both untreated WT and CD103−/− mice, 12%-14% of CD4+ cells expressed CD25. In WT mice, CD28SA expanded these cells to up to 40%, while in striking contrast, cells from CD103−/− mice showed an increase of only up to 25%.

Moreover, CD28SA treatment led to a substantial 2-fold increase of the CD25 expression level of Tregs in WT, but not in CD103−/− mice. Finally, while the FoxP3 expression level of Tregs in WT mice increased 2-fold upon treatment, there was barely any FoxP3 upregulation in CD103−/− mice. These quite distinct CD103-dependent differences of Tregs were highly reproducible and significant.

Together, lack of CD103 negates the stimulatory activity of CD28SA, thus strongly suggesting a role of CD103 in Treg activation. In conjunction with decreased FoxP3 expression in allergic contact dermatitis in CD103−/− mice, it seems likely that CD103 is crucial for Treg activation in general. The mechanistic link between CD103 and FoxP3 furthers our understanding of Treg functions. In addition, it opens new perspectives for selective expansion and activation of potent CD103+ Tregs as a tool for the treatment of immune-mediated disorders.

P123 | Glucocorticoids suppress TLR2/1-induced inflammation, while maintaining TLR-induced host defense programs in human macrophages

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Glucocorticoids, widely used to treat inflammatory conditions in medicine, critically regulate human host defense. In contrast to well-described anti-inflammatory and immune suppressive effects on acquired immunity, recent evidence suggests that glucocorticoids ready innate host defense. In this regard, glucocorticoids were shown to enhance expression of TLR2 on various human cell types. Given a central role of TLR2 in activating macrophage host defense against intracellular pathogens, we studied the effect of glucocorticoids on TLR2 expression and function in primary human macrophages. We found that glucocorticoids upregulated TLR2 mRNA expression, as well as cell surface expression, yet suppressed central components of the TLR signaling cascade. Moreover, glucocorticoids had a much stronger suppressive effect on the TLR2/1 induction of pro-inflammatory cytokines TNF-α and IL-12p40 than on anti-inflammatory IL-10, thereby shifting the macrophage cytokine production towards an anti-inflammatory pattern. At the same time, glucocorticoids did not affect the TLR2/1-induced expression of cathelicidin antimicrobial peptide or lysosome acidification, which are two central host defense mechanisms against intracellular pathogens. In summary, our data suggest that glucocorticoids suppress TLR2/1-induced inflammatory macrophage responses, while TLR2-induced macrophage-mediated host defense programs against intracellular infection stay intact.

P124 | Immune modulatory effects of methyl fumarate-derived iron carbonyl complexes

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Psoriasis or multiple sclerosis (MS) as well as murine models of autoimmune diseases are characterized by the activation of a pro-inflammatory TH1/Th17 cell response. We previously showed that the small molecule dimethyl fumarate (DMF) directly improves these diseases by generating type II dendritic cells (DC) which then in turn induce an anti-inflammatory, interleukin (IL-) 4 dependent Th2 response. The anti-inflammatory and immune modulating activities of DMF result from the depletion of intracellular glutathione and subsequently the accumulation of reactive oxygen species (ROS), the induction of heme oxygenase-1 (HO-1) and the inhibition of STAT1 phosphorylation. This results in the formation of type II dendritic cells characterized by a decrease of the inflammatory cytokines IL-23 and IL-12 and the induction of IL-10. In addition, the emerging anti-inflammatory role of carbon monoxide (CO) as a therapeutic agent for various conditions characterized by hyperactivation of the immune system has become clear. To overcome the difficulties linked to oral CO administration by inhalation of the gas, CO-releasing molecules (CORMs) were developed, which likewise exert their anti-inflammatory potential through activation of heme oxygenase-1 (HO-1). We therefore reasoned to chemically combine fumaric acid and CO in a single molecule and synthesized CO-releasing molecules (CORMs) linked to methyl fumarate (FUMET-CORMs). We then measured the biological activity of the FUMET-CORMs and compared their effects to the single treatment with DMF or CO alone. First results showed that treatment of bone marrow derived dendritic cells (BMDC) with FUMET-CORMs didn't diminish glutathione levels, whereas ROS levels increased substantially. Western Blot analysis and ELISA measurements revealed that treatment with FUMET-CORMs resulted in the induction of HO-1 and the inhibition of STAT1 phosphorylation and subsequently lead to reduced IL-23 and IL-12 levels. Moreover, the inhibitory effects of FUMET-CORMs on the HO-1 and STAT1 signaling pathway occurred at lower concentrations and were stronger as compared to DMF treatment alone. Taken together, our data confirm the concept of combining fumaric acid with CO-releasing molecules as a highly effective therapeutic option by transforming pro-inflammatory dendritic cells to a type II phenotype. Thus, FUMET-CORMs have great potential, e.g. for the treatment of psoriasis or other inflammatory conditions of the skin.

P125 | Platelets induce a regulatory phenotype in CD4+ T cells

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Beside their main function of initiating homeostasis, it has become recognized that platelets are also important players in innate and adaptive immunity through interaction with immune cells. They are rapidly deployed to sites of infection and thus are able to modulate the inflammatory process. Detailed information about the platelet-immune cell interaction in case of inflammation is still elusive. Glycoprotein A repetitions predominant (GARP) was first described on platelets and as an activation marker on the surface of human regulatory T cells (Treg), modulating the bioavailability of TGF-β. Our group has recently shown that GARP is involved in the regulation of peripheral immune responses. The soluble form of GARP (sGARP) has strong anti-inflammatory and regulatory properties in vitro as well as in vivo and leads to induction of peripheral Treg, inhibition of tumor-antigen-specific CD8+ T cells polarization of protumerogenic macrophages.

In this study, we analyzed the effect of platelets on the differentiation and phenotype of CD4+ T cells according to GARP. CD4+ T cells were cocultured together with different ratios of platelets and platelets' supernatant. Herein, platelets led to a Foxp3-induction, anergy and a reduced cytokine production as well as induction of a suppressive phenotype in cocultured CD4+ T cells. These effects were reversed using a blocking anti-GARP mAb in coculture, indicating a GARP dependent induction of Treg in the presence of platelets. Further studies will correlate our findings with clinical data of paraneoplastic thrombocytosis in cancer patients.

In conclusion, our data give evidence that platelets are capable of inducing peripheral Treg (pTreg). Thus, the platelet mediated induction of pTreg could play an important role in diseases like cancer where increased numbers of circulating platelets (thrombocytosis) are associated with bad prognosis and metastasis.

P126 | Systemic and topical triclosan aggravates murine atopic dermatitis

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Triclosan is a common broad-spectrum antibacterial agent used extensively in household, medical and personal products (such as toothpaste). Particularly, it has been used in Germany for decades as topical antiseptic in the management of atopic dermatitis (AD). While there is generally no sensitizing potential attributed to triclosan, recent studies suggest a link between increased urinary levels of triclosan and augmentation of allergic diseases. The exact effects of triclosan on AD remain poorly understood.

Female BALB/c mice were repeatedly treated topically with triclosan at concentrations ranging from 0.75% to 3%, which induced significant concentration-dependent skin irritation. Further, we used the vitamin D3 analogue MC903, which triggered an AD-like skin disease, and we combined the treatment with topical triclosan. We observed a marked deterioration of the MC903-induced AD-like skin disease after additional triclosan treatment. To dissect the immune response involved in this reaction, cellular infiltrates in the affected skin as well as draining lymph nodes were analyzed by immunohistochemistry, flow cytometry and qPCR with regard to T cell subsets, innate lymphoid cells and major cytokines involved. The identical negative effect of triclosan on AD-like skin disease was also reproducible after systemic application of triclosan.

In conclusion, our findings suggest that triclosan does not only possess antibacterial and antifungal properties but also causes immunomodulatory effects in AD. Translational studies are urgently needed for evaluation of a pathogenic role of triclosan in human AD.

P127 (OP05/02) | Dynamics of neutrophil extracellular trap (NET) formation

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Introduction: Neutrophils can catch and kill pathogens by expelling a fibril network made from their own DNA (Neutrophil Extracellular Traps, NETs). NETs are also involved in the pathogenesis of autoimmune and inflammatory diseases such as lupus erythematosus, psoriasis or rheumatoid arthritis. During this process (suicidal NETosis), cells rearrange their contents profoundly. Within a few hours, the cytoskeleton disassembles, the cell depolarizes and rounds up. Simultaneously, the nuclear chromatin first expands to fill the whole cell and is then released into the extracellular space, ultimately leaving the neutrophils to die. The mechanistic basis of these fundamental processes, however, remains poorly characterized. The aim of this project is to understand which biophysical aspects govern NETosis, how the chromatin of the cell is rearranged during NETosis and how the DNA finally leaves the cells (active or passive process).

Methods: Human neutrophils were activated with phorbol 12-myristate 13-acetate (PMA), bacterial lipopolysaccharides (LPS) or calcium ionophore. DNA decondensation and cell membrane reorganization during NETosis were observed in real time with conventional fluorescence and confocal laser scanning microscopy (CLSM) as well as reflection interference contrast microscopy (RICM). To determine whether NETosis involves passive/active mechanisms, inhibitors of enzymatic activity (sodium azide, EDTA) were added at different time-points and experiments were performed at different temperatures (23.5°C, 37°C, 40°C). Changes of the mechanical properties were observed by time-resolved atomic force microscopy (AFM) measurements.

Results: NETosis has three clearly distinct phases: P1) Neutrophil activation; lobulated nucleus, P2) Decondensation of the chromatin within the confines of the cell membrane; cell rounding, P3) Rupture of the cell membrane; release of chromatin into the extracellular space.

P1 is strongly dependent on enzymatic activity and temperature, suggesting active mechanisms. In contrast, P2 appears to be governed mainly by passive mechanisms driven by the entropic pressure of chromatin swelling. Duration of P2 correlates directly with cell size but is largely independent of temperature and metabolic inhibitors. P3 occurs directly after the cell has reached maximum circularity and minimum stiffness, as determined by AFM. The membrane ruptures at a biomechanically predetermined breaking point.

Conclusions: In this first biophysical characterization of NETosis, we define three distinct phases, which are differentially orchestrated by active or passive mechanisms, respectively. It is likely that the molecular players and processes identified through this work will have implications for general principles of structural re-organization and membrane dynamics of cells.

P128 | In vitro formation of neutrophil extracellular traps (NETs) is inhibited by the presence of serum and serum albumin in culture media

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Introduction: Neutrophils can bind and kill pathogens by the expulsion of "Neutrophil Extracellular Traps" (NETs), which are composed of chromatin and granular proteins. NETs have also been implicated in autoimmune diseases including lupus erythematosus, psoriasis or rheumatoid arthritis. In vitro approaches to study NET formation (NETosis) usually involve neutrophil stimulation with activators such as phorbol 12-myristate 13-acetate (PMA), lipopolysaccharides (LPS) or calcium ionophores (CaI). However, the variability of experimental conditions renders any comparison of results between research groups impossible and complicates their interpretation. One prominent example is the supplements added to the media: In human experiments, either heat inactivated serum (hiFCS, 0.5% to 10%) or serum albumin (HSA; 0.2% to 2%) was added to the medium, while murine neutrophils were largely studied in serum-free medium. These differences may greatly influence the outcome of NET-experiments, as albumin can bind proteins like LPS. Here, we evaluated the influence of different media supplements on NET formation of human neutrophils to lay a foundation for the unification of experimental conditions involving NETs.

Methods: Human neutrophils were isolated from healthy donors and resuspended in RPMI medium containing 10 mM Hepes and either 0.5-2% hiFCS, 0.5% HSA or no supplement. NETosis was induced by PMA, LPS isolated from Pseudomonas aeruginosa or CaI. NETs and decondensed nuclei were quantified using the DNA dye Hoechst.

Results: The addition of hiFCS resulted in a dose-dependent inhibition of NETosis; serum-free medium yielded the highest NET-rate after stimulation with LPS, CaI or PMA, respectively. The addition of 0.5% HSA to the medium efficiently prevented NET formation following stimulation with LPS and CaI and resulted in a trend towards a reduction of NETosis after PMA stimulation.

Conclusion: Serum components such as HSA and hiFCS inhibit NET-formation to different degrees at the concentrations typically found

in the literature concerning in vitro NETosis experiments. Thus, the choice of media supplements greatly determines the outcome of in vitro experiments on NET-formation and should be unified to allow for a better comparison between results.

P129 | Type 2 innate lymphoid cells act as regulators of type I driven TNCB contact hypersensitivity

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The role of innate lymphoid cells (ILCs) in allergic contact dermatitis (ACD) has not adequately been addressed. We sought to investigate quantitative changes and functional relevance of ILCs during the elicitation phase of hapten-induced contact hypersensitivity (CHS). EomesGfp/+ x Rorc(γt)-CreTg x Rosa26RYfp/+ reporter mice were sensitized and challenged with the hapten TNCB. Ear swelling responses, ILC numbers and cytokine production were measured at different time points. For functional analysis sorted T-cells from TNCB-sensitized donor mice (CD90.1) were adoptively transferred i.v. in Rag1−/− mice (CD90.2). Rag1−/− mice recipient mice were treated with either a CD90.2-specific or isotype mAb before T-cell transfer and allergen challenge. In addition CHS was performed in Rorα+/floxIl7rCre mice, which selectively lack ILC2. The quantitative analysis of total cell numbers revealed early increases of natural killer (NK) cells in skin and skin draining lymph nodes (SDLN) 24 hours after allergen challenge, corresponding to the highest ear swelling response and leukocyte infiltrate in the skin. These cells produced high amounts of the type 1 cytokines interferon gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-a. ILC1, 2 and 3 showed a delayed increase in total numbers starting from 48 hours after allergen challenge. ILC2 cells displayed an activated phenotype reflected by increased ICOS expression. Total ILC depletion through CD90.2 mAb administration resulted in a significantly enhanced ear swelling response as compared to isotype control treated mice. Finally, Rorα+/floxIl7rCre mice, that selectively lack ILC2 cells, also displayed increased ear swelling responses after allergen challenge compared to WT mice. In conclusion, our data support the concept of NK cells as main innate pro-inflammatory players and suggests that simultaneously activated ILC2 counteract as regulators in the type 1 dominated immune response of CHS.

P130 | Neutrophil activation in psoriasis is associated with enhanced mechanical deformation

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Psoriasis is a chronic inflammatory skin disease characterized by infiltrating immune cells which are recruited into the skin by a complex network of chemokines. We showed that the chemokine CXCL16 is upregulated in psoriatic skin. CXCL16 exerts its function by ligation of its receptor CXCR6 and recruits CXCR6+ CD8+ T cells into psoriatic skin. Interestingly, neutrophils in blood of psoriatic patients also express CXCR6. CXCL16 induced neutrophil migration and enhanced the chemotactic response of neutrophils to CXCL8/IL-8, which is a potent neutrophil chemoattractant in psoriasis. As transmigration of cells into tissue requires mechanical deformation we were interested in the morphological and mechanical characteristics of neutrophils in psoriatic compared to healthy individuals.

Using real-time deformability cytometry allows analysis of cell deformation in real time at rates of 1000 cells/sec, approaching the throughput of conventional flow cytometers. We observed that untreated neutrophils from patients with psoriasis were larger and had a more irregular shape than neutrophils from healthy controls which can be interpreted as a sign of activation. Upon continuous application of shear stress in a microfluidic channel constriction neutrophils from psoriatic patients showed a higher deformation compared to neutrophils from healthy individuals. Additional stimulation with IL-8 even enhanced deformation in psoriatic as well as control neutrophils. In contrast, an increase of deformation upon CXCL16 ligation was only recorded for neutrophils from blood of healthy individuals whereas CXCL16 had no additional effect on deformation of neutrophils from psoriatic patients. In conclusion, our data allows to interpret that neutrophils from blood of patients with psoriasis are activated and more prone to deformation upon shear stress which is additionally increased by IL-8. The enhanced mechanical deformability due to CXCL16 and IL-8 could likely favor transmigration into tissue leading to psoriatic inflammation. These findings suggest that the chemokines CXCL8/IL-8 and CXCL16 not only mediate migration of neutrophils but already alter their mechanical properties in blood.

P131 | Influence of neutrophil granulocytes treated with extracorporeal photopheresis on cutaneous fibrosis

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Background and objective: Chronic graft-versus-host disease (GVHD) is a common side effect after allogeneic human stem cell transplantation and can lead to severe fibrosis of the skin. Besides immunosuppressive therapy, photo-chemotherapy by extracorporeal photopheresis (ECP) is used as an immunomodulatory therapy for GVHD with low side effects. However, the mode of action of ECP has not been completely understood, yet. So far, clinical activity of ECP has mainly been attributed to mononuclear cells. In a recent study, we could show that polymorphonuclear cells (PMN) are the main leukocyte fraction treated during ECP. To further characterize the role of PMN in ECP, we now established an in vitro model to examine the effects of ECP-treated neutrophil granulocytes on cutaneous fibrosis.

Methods: PMN were isolated from peripheral blood of healthy donors and were treated with 2 J/cm2 UVA after addition of 340 ng/mL 8-methoxypsoralen (8-MOP). A transwell migration assay was performed in 24 well-plates for 3 hours with treated and untreated PMN. Fibroblasts were cultivated from skin of healthy donors and were co-cultured for 48 hours with neutrophils. Neutrophils in these co-cultures were either used untreated or after treatment with 8-MOP and UVA in the presence or absence of TGF-beta at different ratios, as TGF-beta induces fibrosis. Smooth muscle actin (SMA)-expression of fibroblasts was used as a surrogate marker for fibrosis. The level of fibroblast differentiation was detected via SMA-staining and fluorescence microscopy. SMA mRNA expression was also assessed by PCR.

Results: After treatment with 8-MOP and UVA, PMN showed the same migratory properties in a transwell assay as untreated PMN. At a ratio of 1:1 (neutrophils: fibroblasts), a significantly higher number of SMA-positive fibroblasts was detected after 48 hours of co-incubation with untreated neutrophils compared to cultures containing only fibroblasts. Within co-cultures with chemoirradiated PMN, a slight but lower increase in SMA-positive fibroblasts was observed. TGF-beta upregulated SMA in fibroblasts. When TGF-beta was present in the co-cultures, chemoirradiated PMN reduced the number of SMA-positive fibroblasts significantly while untreated neutrophils did not affect SMA expression significantly. Concordantly, fibroblasts co-cultured with chemoirradiated neutrophils also showed a significantly decreased production of SMA-RNA.

Conclusion: Neutrophils show pro-fibrotic properties which can be significantly reduced by chemoirradiation with 8-MOP and UVA. In addition, chemoirradiated neutrophils reverse TGF-b-induced fibrosis in vitro. These findings suggest that the treatment of neutrophils with ECP might be able to suppress GVHD-induced fibrosis. Further translational and murine studies are necessary to unravel the mechanisms underlying these findings.

P132 | Detection of neutrophil extracellular traps in human immune complex vasculitis

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Immune complex vasculitis (ICV) is an inflammation of blood vessels that mainly affects small blood vessels. Decisive initial steps in the pathogenesis are the deposition of immune complexes at the vessel walls followed by neutrophil accumulation and activation which then leads to destruction of blood vessels. However it is unknown how and which cytotoxic components cause this vessel damage. Stimulated neutrophils produce extracellular structures called neutrophil extracellular traps (NETs). NETs are filaments of decondensed chromatin associated with cytotoxic proteins like histones and myeloperoxidases (MPO) and have been implicated in autoimmunity and tissue injury. Interestingly, immune complexes stimulate neutrophils for NETosis.

The aim of this study was to investigate the impact of NETs from human neutrophils, stimulated with immune complexes, on vessel damage during ICV in vitro and in vivo.

To analyze NET concentration and NET-bound proteins in vitro immune complexes were produced and co incubated with freshly isolated human neutrophils either from healthy donors or from vasculitis patients. NETs concentration was measured with a fluorescence dye after inducing NETosis. NET-bound proteins were analyzed using immunofluorescence staining and quantified by image processing. The impact of isolated NETs on endothelial cells was measured with a cytotoxicity assay. In vivo human tissue samples of ICV patients were analyzed for NETs and its cytotoxic components by immunofluorescence staining. For this reason histamine wheals and skin with lesion from ICV patients were used to detect early and late stage of vasculitis, respectively.

We confirmed that isolated human neutrophils can be stimulated by soluble immune complexes to release NETs. When neutrophils from healthy donors were co incubated with immune complexes in vitro they show formation of NETs after 4 hours. Additionally it was observed that neutrophils from ICV patients are overresponsive. This was revealed by a comparison of PMA and immune complex stimulated neutrophils of vasculitis patients and healthy donors. The result demonstrates higher fluorescence intensity for ICV patients reflecting a higher NETosis rate. Also neutrophils from ICV patients revealed a higher amount of MPO bound to NETs than those from healthy donors after stimulation with PMA.

In relation to human endothelia cells an increasing cytotoxicity was measured with raising NETs concentrations.

Seeking in vivo evidence of NET formation, we analyzed a panel of skin needle biopsies from subjects with vasculitis and found typical components of NETs i.e. DNA in combination with histones and neutrophil granule proteins which are located in close proximity to neutrophil infiltrates in the skin.

We conclude that immune complexes both induce NETosis and also bind NETs, thus indicating that toxic MPO is concentrated near endothelial cells and that way involved in its damage.

P133 (OP05/05) | Role of histone H2A deubiquitinase Mysm1 in immune cell development, T helper cell differentiation and autoimmunity

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Myb-like SWIRM and MPN containing domain (Mysm1, also termed 2A-DUB) is a histone modifying enzyme that catalyzes the deubiquitination of lysine 119 (K119) on core histone H2A. Based on recent studies and own data revealing functions of Mysm1 in lymphopoiesis, early T cell development and changes in T cell subpopulations in Mysm1−/− mice, we hypothesized that Mysm1 might affect T helper cell differentiation and autoimmunity.

In the present investigation, we therefore analyzed Treg and Th17 development in Mysm1-deficient mice in more detail. Our preliminary data indicated significant upregulation in the expression of Foxp3, the master transcription factor of Tregs, as well as increased levels of CD25 expression, a marker for both Tregs and activated T cells, in thymi of Mysm1−/− mice. Due to the altered natural Treg development in these mice, we next focused on peripheral T cell differentiation. Despite the severe reduction of T and B cells, peripheral T cell proliferation was not consistently impaired. Also, in line with normal peripheral T cell activation, Mysm1−/− T cells upregulated the early activation markers CD69 and CD25 upon activation to similar extent as wild-type T cells. Interestingly, basal Foxp3 expression in peripheral Mysm1−/− T cells was significantly higher in comparison to wild-type T cells. To further evaluate the potential role of Mysm1 in Th17/Treg specification, in vitro differentiation assays were performed, in which sorted splenic T cells were differentiated to Tregs under appropriate stimulating conditions and analyzed for expression of Foxp3. In agreement with our hypothesis, Mysm1−/− T cells demonstrated higher induction of Foxp3 expression in comparison to the wild-type T cells. Current work is focusing on functional experiments such as the in vitro Treg suppression assay.

Furthermore, in order to dissect whether defective lymphoid development in absence of Mysm1 is caused by an intrinsic requirement of Mysm1 in lymphoid lineages or earlier defects in hematopoietic stem cells (HSCs), a new mouse strain (CD127-Cre:Mysm1tm1a) with lymphoid-specific deletion of Mysm1 in CD127/ IL-7Rα-expressing cells is currently being generated. The newly generated CD127-Cre:Mysm1tm1a mice will be systematically analyzed for lymphoid development and differentiation in primary and secondary lymphoid organs. To further investigate the role of Mysm1 in autoimmunity, a mouse model of imiquimod-induced psoriasiform dermatitis is applied. Among the phenotypic abnormalities of the Mysm1−/− mice skin atrophy and altered melanocyte development have been detected. Therefore, skin immunity is being studied in the context of inflammation and autoimmunity in conventional and conditional Mysm1 mutant mice.

In summary, the investigation of Mysm1 functions in Treg/Th17 differentiation will improve our understanding on the role of histone modifications in lineage specification of T cell subsets and aid the long-term goal of developing novel therapies for disease conditions such as autoimmunity, infections and cancer.

P134 | Immunomodulatory effects of mesenchymal stem cells on macrophage activation

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Macrophages have a crucial role in all the phases of wound healing: inflammation, proliferation and remodeling. Persistent unrestrained activation of inflammation-perpetuating macrophages is causal for non-healing of chronic wounds. Macrophages occur within a spectrum ranging between two main phenotypes, classically activated pro-inflammatory type M1 macrophages and alternatively activated anti-inflammatory type M2 macrophages. The shift from pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages is severely disturbed in a variety of M1 macrophage dominated chronic wound disorders.

Mesenchymal stem cells (MSCs) are characterized by their differentiation potential into multiple histogenetically distinct cell types. In addition they are endowed with the capacity to modulate immune cells, among them M1 macrophages, and in consequence dampen unrestrained inflammation at the wound site by paracrine factors. To uncover molecular targets that might be involved in down regulation of M1 activation by MSCs under inflammatory conditions, we employed qPCR and microarray analysis and specific ELISAs of supernatants from co-cultured MSCs and macrophages, which before analysis were separated by FACS. The transcriptome analysis showed that MSCs promote the conversion from M1, with high expression of the M1 markers TNF-α and IL-12p40, to M2 macrophages with increased expression of M2 markers like IL-10, IL-1RA and CD206. These findings were confirmed by qPCR and specific ELISAs. A comprehensive unbiased microarray analysis furthermore uncovered a variety of previously unreported molecular targets that might be instrumental in the MSCs control of the unrestrained activation of M1 macrophages. Among these new targets we identified osteopontin, an important multi-domain protein with migration enhancing and possibly inflammation modulating properties. Interestingly, osteopontin expression was significantly down-regulated by MSCs when co-cultured with pro-inflammatory M1 macrophages.

To further substantiate the notion that apart from cytokines also components of the extracellular matrix may modulate MSCs activity and their immunomodulatory function, we explored whether heparan sulfate (HS), a main constituent of the extracellular matrix, is able to modulate the immunosuppressive potential of MSCs. MSCs cultured in the presence of HS were stimulated with LPS and IFN-γ to mimic inflammation. Under these conditions, a significant decrease

in expression as well as secretion of the pro-inflammatory IL-6 was found. This is particularly interesting as IL-6 can perpetuate pro-inflammatory M1 macrophages via NFκB activation. These results will help to gain knowledge on the conditions for MSCs employed for clinical use and may improve the effect of MSCs applied to the hostile pro-inflammatory microenvironment of chronic wounds.

P135 | Regulatory T cells suppress the myeloid cell-dependent inflammation and blistering in pemphigoid diseases

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Pemphigoid diseases (PD) are a group of rare autoimmune blistering skin diseases. In most pemphigoid diseases, autoantibody binding alone is not sufficient to lead to clinical disease manifestation. For the later, myeloid cells are a prerequisite but the impact of other cell types like T cells is not investigated very well. It is long known that BP patients had lower Treg numbers in the skin and circulation, while Th17 cells were found more frequently in the skin of patients. Here, the role of Treg on skin inflammation and blistering in PD were assessed in detail using antibody transfer-induced bullous pemphigoid (BP) and epidermolysis bullosa acquisita (EBA) mouse models and "depletion of regulatory T cell" (DEREG) mice which were injected with diphtheria toxin (DT) to reduce the amount of circulating Treg. Compared to DT injected wild-type controls, Treg depletion in DEREG mice led to a significantly, approximately 2-fold, increase in skin inflammation and blistering. This was accompanied by an increase in leukocyte dermal infiltration, while IgG and C3 deposition at the dermal-epidermal junction were not affected. Corresponding observations, with an even more pronounced clinical phenotype, were made in antibody transfer-induced EBA. To further analyze possible mechanistic effects, cytokine gene expression in lesional skin of wild-type or Treg-depleted mice was evaluated. Unexpectedly, the expression of innate cytokines such as IL-1ß and TNF known as prominent cytokines in EBA skin lesions did not differ between wild-type and Treg-depleted mice. Instead, the Th1 cytokine IFN-γ and the Th2 cytokines IL-4, IL-10 and IL-13 were significantly increased in Treg depleted mice. In addition, the expression of the T cell attracting chemokine CXCL-9 was evaluated in lesions of Treg depleted mice. Therefore, the increased dermal infiltrate observed in DEREG mice after PD induction, is most likely driven by IFN-γ and CXCL-9 whereas the anti-inflammatory properties of the other differentially expressed cytokines, especially IL-10, are not sufficient to prevent blistering, but rather represent an insufficient counter mechanism. These data correlate with increased IFN-γ serum levels that indicate a general inflammation in Treg depleted mice.

In summary, we demonstrate that Tregs control myeloid cell-mediated skin inflammation in experimental PD, and that this property of Treg is most likely mediated by modulation of cytokine production in the skin, which regulates leukocyte extravasation into the skin and/or increased leukocyte activation.

P136 (OP01/01) | Myeloid cell-specific STAT3 signaling regulates bleomycin-induced skin fibrosis via inhibition of an autocrine TGF-beta loop

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Skin repair after mechanical injury is characterized by the replacement of granulation tissue with extracellular matrix. Pathological healing conditions, as associated with chronic venous diseases, diabetes mellitus or autoimmunity, often cause excessive accumulation of fibrous connective tissue leading to fibrosis and organ malfunction. Inflammation is considered a key factor driving the development and progression of the fibrotic diseases. However, detailed understanding how elements of the inflammatory cascade induce and sustain a fibrotic response is elusive. In this study we aim to unravel the functional impact of macrophage activation during the development of skin fibrosis. We examined myeloid-cell restricted signaling of Signal transducer and activator of transcription 3 (STAT3), a transcription factor implicated in the resolution of inflammatory responses.

To examine the functional impact of STAT3-mediated macrophage activation during the course of skin fibrosis, we generated myeloid cell-specific STAT3 deficient mice (STAT3MKO) and analyzed bleomycin-induced skin fibrosis. We identified myeloid cell-restricted STAT3 activation as important suppressor of skin fibrosis. Increased tissue fibrosis in bleomycin-induced lesions in STAT3MKO mice was characterized by increased collagen deposition and pro-fibrotic factors including COMP. Whereas the absolute number and the percentage of macrophages and neutrophils within fibrotic lesions were comparable in STAT3MKO vs control mice, transcripts of several mediators controlling autocrine and paracrine TGF-beta1 activity were differentially expressed in macrophages isolated from fibrotic skin lesions in STAT3MKO vs control mice. Bleomycin-mediated injury in control mice resulted in increased expression of IL-10, SOCS3, and decorin in macrophages, all factors that previously have been reported to inhibit TGF-beta1 signaling and/ or the development of fibrosis. In contrast, in STAT3-deficient macrophages, expression of these genes was significantly attenuated when compared to controls, proposing their functional role in the accelerated fibrotic response in STAT3MKO mice. Consistently, TGF-beta1 transcripts and downstream targets such as pSmad2 were significantly increased in lesional STAT3-deficient macrophages and the fibrotic lesion, respectively. To corroborate our in vivo findings suggestive for increased TGF-beta1 activity in STAT3MKO mice, we investigated macrophage-fibroblasts cocultures. Notably, these experiments confirmed IL-10-STAT3-mediated suppression of TGF-beta1 expression in macrophages, and alleviation of this suppression by STAT3-deficiency. In fact, co-culture experiments of STAT3-deficient macrophages with dermal fibroblasts

resulted in increased CTGF expression in fibroblasts that could be repressed by TGF-beta1 blocking antibodies.

Conclusively, our findings identify IL-10-mediated activation of STAT3 in macrophages as important suppressor of bleomycin-induced skin fibrosis. Our findings provide new mechanistic insights into the macrophage-fibroblast crosstalk and uncover novel therapeutic targets to limit pro-fibrotic skin diseases.

P137 | The NCOA/PPARγ axis modulates Treg/Th17 and γδ T cell plasticity in psoriasiform skin inflammation and psoriasis

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The importance of the IL-23/IL-17 axis in human inflammatory skin diseases is impressively reflected by the high efficacy of modern biologicals targeting these cytokines. Based on data from the CD18hypo PL/J mouse model of psoriasiform dermatitis, we first systematically quantified the presence of CD3+TNF-α+ and CD3+IL-17+ T cells, and of Foxp3+IL-17+ double-positive T cells among IL-17 producing cells in inflamed skin of human psoriasis patients by immunofluorescent analyses of biobank material to further confirm the concept of Th cell plasticity and conversion of regulatory T cells into Th17 cells in skin inflammation. In order to uncover novel potential mediators affecting the Treg/Th17 balance, we performed in-depth global gene expression studies and functional analyses with CD90.1+ T cells isolated from lesional vs healthy murine CD18hypo PL/J skin. Data were compared with affected mice treated with fumaric acid esters (FAE), representing a therapeutic option for psoriasis patients that may influence the transcription factor balance in T cells, and subsequently verified in newly generated mutant mice.

In global gene expression analyses, we could identify peroxisome proliferator-activated receptor gamma (Pparγ) – previously implicated in negative regulation of Th17 differentiation in other autoimmune models – and co-regulators of Pparγ to be among the genes most significantly down-regulated in murine CD90.1+ T cells during skin inflammation. In line with previous data, apart from Pparγ, mRNA expression levels of redox-modulating enzymes, including glutathione peroxidases (Gpx2, -4, -18) and superoxide dismutases (Sod1, Sod3) responded well to treatment with FAE, whereas expression of other regulators such as Hif-1α, Nrf2, and hemoxygenase was not significantly altered in murine T cells after 14 days of FAE treatment.

To further evaluate the functional role of the NCOA/PPARγ axis in Treg/Th17 and γδ T cell plasticity, Ncoa-knockout mice were analyzed for changes in T cell development and inflammation. While overall T cell development was grossly normal in Ncoa-deficient mice, preliminary data indicated increased skin inflammation and presence IL-17 producing cells upon treatment with Toll-like receptor 7/8 (TLR7/8) agonist Imiquimod (IMQ). Skin, lymph nodes, and spleens from Ncoa-deficient mice treated with either IMQ or vehicle following established protocols were further analyzed for total cellularity, presence of IL-17-producing cells, and overall cytokine production in comparison with wild-type littermates as well as in in vitro Treg/Th17 differentiation assays. Furthermore, binding of Pparγ co-regulators to the Rorc promoter was detectable in chromatin immunoprecipitation (ChIP) assays, substantiating the potential role of these transcriptional co-regulators in Th17 differentiation.

In conclusion, T cell plasticity and the effect of FAE on the Foxp3/Rorγt balance in skin αβ and γδ T cells in the CD18hypo PL/J psoriasis model in vivo and in vitro and in human psoriasis may at least in part depend on the NCOA/PPARγ axis. Our data suggest that PPARγ and its co-regulator NCOA are required to stabilize regulatory properties of T cells – and potentially other immune regulatory cells – and in the course of skin inflammation, down-modulation of the NCOA/PPARγ axis may contribute to an overall conversion and induction of an inflammatory T cell status. This newly identified transcriptional modulation of skin T cell plasticity and mode of action of redox-modulatory drugs may help to further improve therapies of IL-17 mediated inflammatory diseases and to "reprogram" an aberrant immune system in autoimmune conditions of the skin and other organs.

P138 | Chymase-CreMcl-1fl/fl mice exhibit reduced numbers of mucosal mast cells

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Mast cells (MCs) are potent inflammatory cells that are found predominantly at the interface between the host and the external environment. MCs play an important role in host defense responses to bacterial and parasite infections. In mice, two main types of MCs have been described: connective tissue MCs (CTMCs) and mucosal MCs (MMCs). MMCs, under physiological conditions, are found at relatively low numbers in most mucosal tissues, but expansion of MMC populations can be induced in a T cell-dependent manner. However, the knowledge about the biological functions of MMCs is limited due to the lack of suitable models to investigate MMCs in vivo. We, therefore, have generated a new mouse model that exhibits a specific deficiency in MMCs, thus allowing for the investigation of MMCs in vivo. It has been previously reported that Cre expression driven by a chymase promotor correlates to mature resident mucosal MCs. We mated chymase-Cre transgenic mice with mice bearing a floxed allele of the myeloid cell leukemia sequence 1 (Mcl-1), which encodes for an intracellular antiapoptotic factor in MCs. When comparing Chy- Cre;Mcl-1 fl/fl and wild-type mice, we found equal numbers of connective tissue type MCs such as peritoneal or skin MCs as well as similar proliferation and differentiation rates in bone morrow-derived cultured MCs (BMCMCs) in histological and flow cytometric analyses. In contrast, we observed a significantly reduced number of MCs by quantitative histomorphometry in the uterus of Chy-Cre;Mcl-1 fl/fl mice (0.50.5/ high power field

(HPF), n=6) compared to wild-type mice (7.71.5/HPF, n=6, *P*<.001). Also, we found markedly reduced MCs numbers in the stomach of Chy-Cre;Mcl-1 fl/ fl mice (0.80.7/HPF, n=12) compared to wild-type mice (6.41.6/HPF, n=12, *P*<.05). Taken together, our results show that this new mouse model presents with markedly reduced numbers of MCs in mucosal tissues, i.e. stomach and uterus. Therefore, the Chy-Cre;Mcl-1 fl/fl model could become a useful tool for the investigation of the pathophysiological functions of MMCs in vivo.

P139 | Defective clearance of intracellular nucleic acids enhances sensitivity to extracellular stress and predisposes to autoimmunity

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Cytosolic nucleic acid restriction is essential to prevent immune activation by innate intracellular nucleic acid sensors. The DNA exonuclease TREX1 is located in the endoplasmic reticulum where it safeguards the cytosol against DNA accumulation and prevents innate immune responses and autoimmunity. Mutations impairing the function of TREX1 lead to accumulation of unrestricted DNA in the cell associated with type I interferon induction by cell intrinsic innate immune activation. The encephalopathy Aicardi-Goutieres syndrome characterized by symptoms of autoimmune disease and systemic lupus erythematosus belong to the disease spectrum associated with mutations in TREX1. TREX1 deficient cells of such patients harbor increased DNA damage and show continuous activation of the DNA damage response. Lupus patients with TREX1 mutation were reported to be sensitive to the environmental trigger sun light which can induce disease flares. Analysing the effect of external triggers we found that TREX1-deficient fibroblasts showed enhanced DNA damage and a stronger elevated DNA damage response compared with wild-type cells upon exposure to UV irradiation or reactive oxygen species. This was associated with a reduced proliferation rate and an upregulation of type I interferon induced genes. Enhanced expression of interferon induced genes facilitated the production of type I interferon upon exposure to a viral mimics poly(I:C) and solar-simulated UV irradiation. In conclusion, we show that environmental stressors enhance DNA damage in patients with genetic defects in nucleic acid metabolism and lead to an upregulation of type I interferon induced genes which favor type I interferon induction and development of autoimmunity.

P140 | Macrophage migration inhibitory factor (MIF) promotes TH17 cell-driven psoriasiform dermatitis

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Macrophage Migration Inhibitory Factor (MIF) is a unique protein that combines properties of cytokines, enzymes, hormones, and chaperones, and exerts pleiotropic effects. In sum, its effects are mostly considered pro-inflammatory. Accordingly, MIF has been suggested to promote the pathogenesis of multiple inflammatory disease in diverse organs. Thus, it is elevated in the serum of psoriasis patients, and functional polymorphisms in the MIF gene are associated with enhanced susceptibility to psoriasis. The significance of these findings for the pathogenesis of psoriasis, however, has remained elusive. We therefore addressed the role of MIF in psoriasis by examining Mif−/− mice in the Aldara- (AIPD) and in the IL-23-induced psoriasiform dermatitis model. Genetic deficiency in MIF reduced clinical severity of psoriasiform skin inflammation in the AIPD and in the IL-23-induced model by approximately 50%. On the histopathological level, psoriasiform skin lesions of Mif−/− mice showed less T cell and macrophage infiltration in the skin. Likewise, epidermal proliferation as well as dermal neoangiogenesis were markedly attenuated.

Our results suggest a significant role for MIF in the pathogenesis of plaque psoriasis and highlight it as a new therapeutic target in the treatment of the disease that may be selectively applied in those patients with high expression MIF risk alleles. With exogenous glucocorticoids known to upregulate MIF, the inhibition of MIF may have particular application when used adjunctly with topical corticosteroids.

P141 | 12/15-lipoxygenase aggravates psoriasiform dermatitis

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Psoriasis is chronic inflammatory disease of the skin. In recent years, it has been associated with metabolic diseases, including cardiovascular diseases, diabetes, obesity, and arterial hypertension. Consequently, psoriasis has been proposed as independent risk factor for the metabolic syndrome. However, the common molecular mechanisms driving both diseases in parallel are still largely elusive.

12/15-lipoxygenase (12/15-LO), an enzyme exerting pleiotropic actions, has been suggested to be an important driver of the metabolic syndrome, promoting the pathogenesis of cardio-cerebrovascular disease, diabetes, obesity, arterial hypertension, and non-alcoholic steatohepatitis. The enzyme and its major products, 12(S)- and 15(S)-HETE are also elevated in psoriatic lesional skin as well as in the urine of psoriasis patients. However, the functional significance of 12/15-LO in psoriasis has never been addressed.

We therefore hypothesized that 12/15-LO may play a role in the pathogenesis of psoriasis. To address this question, we examined

12/15-LO-defcient (Alox15−/−) mice in three complementing mouse models of psoriasis, the Aldara-, the IL-23-, and the TPA-induced psoriasiform dermatitis model. Deficiency in 12/15-LO ameliorated skin inflammation in all three models of psoriasiform dermatitis. On the histopathological level, psoriasiform skin lesions exhibited attenuated cell infiltration and reduced epidermal hyperproliferation. Subsequent mechanistic studies suggested that 12/15- LO promotes psoriasiform skin inflammation, among others, by reinforcing the expression of IL-6 in psoriatic skin lesions.

Our results highlight 12/15-LO as common promoter of psoriasis and metabolic diseases, which may partially explain the close association between these two diseases. 12/15-LO is therefore a potential drug targets for the simultaneous treatment of both diseases.

P142 (OP04/06) | Psoriatic inflammation is initiated by DAMP/DNA complexes on plasmacytoid dendritic cells via RAGE

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A broad range of studies has highlighted the central role for the receptor for advanced glycation end-products (RAGE) signaling in inflammation by sensing damage-associated molecular patterns (DAMP). However, the relative contribution of RAGE and its ligands to the pathogenesis of the prototypic inflammatory skin disease psoriasis remains largely elusive. We show here that mice deficient for RAGE developed a diminished psoriasiform response to topical imiquimod-induced chronic inflammation. Stimulation of keratinocytes with imiquimod resulted in a RAGE-independent release of S100B and HMGB1, both members of the DAMP. In an in vitro system, we demonstrated that S100B and HMGB1 in complex with self-DNA activate CD11c+ plasmacytoid dendritic cells via RAGE to secrete high amounts of IFN-α indicating a RAGE-dependent epidermal-innate immune cell crosstalk in psoriasis. Finally, the defective inflammatory phenotype of Rage-deficient mice was rescued by intradermal injections of recombinant IL-23 suggesting IL-23 as a putative target of RAGE. Our data point towards a central role for RAGE signaling in the IL-23/TH17 axis of psoriasis, therefore representing a potential target for new therapeutic strategies.

P143 | Interface dermatitis shows a distinctive molecular signature independent from individual disease background

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Inflammatory skin diseases (ISDs) are highly heterogeneous in terms of clinical appearance, histological architecture, pathogenesis and underlying triggers. However, certain histological criteria are consistently shared by several ISDs and are therefore an ideal model for investigation of general mechanisms of skin inflammation. Interface dermatitis (ID), characterized by a dense lymphocytic infiltrate at the dermo-epidermal junction in combination with apoptotic basal keratinocytes, is a perfect example for this issue, as it appears in inflammatory skin diseases, like lichen planus (LP), as well as in autoimmune diseases, like cutaneous lupus erythematosus (CLE). In this study 18 cases of either CLE (n=6) or LP (n=12) were analysed using a histology score based on 24 objective criteria, thereby allowing a disease independent ranking of ID severity. Furthermore, we performed whole genome expression analysis of lesional and autologous non-lesional skin biopsies identifying genes correlating with the individual ID severity. A high regulation of chemokine (CXCL2, CXCL13) and T cell receptor genes (CD247) as well as a contribution of the JAK/STAT pathway, revealed by induced network modules analysis, characterizes the molecular signature of ID. This study is a new approach to investigate inflammatory skin diseases using objective histological phenotyping instead of conventional disease terminology, thus demonstrating an advanced way to interpret gene expression data and to understand key pathways of skin inflammation.

P144 | Regulated expression of CD73 by subsets of dendritic cells in skin is crucial for modulating contact hypersensitivity reactions in mice

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The ecto-5′-nucleotidase CD73 converts extracellular adenosine monophosphate, which is a product of ATP degradation by CD39, to adenosine (ADO). Because ADO has well established anti-inflammatory effects, expression of CD73 by different types of skin cells may be crucial for the outcome of contact hypersensitivity (CHS) reactions.

At first we analysed expression of CD39 and CD73, respectively, by different subsets of skin dendritic cells (DCs) and found ubiquitous expression of CD39 on all defined DC subsets. In contrast, CD73 was expressed by only 3%-8% of CD207+ Langerhans cells (LC) and other dermal DCs. Similar expression patterns were observed in skin migrating (sm) DCs in the lymph node under steady state conditions. Application of the hapten TNCB induced surface-expression of CD73 in all smDCs populations, resulting in 20%-30% of cells CD73+. This increased expression of CD73 was persistent, as it was

also detectable in smDC 24-48 hours after sensitization. Next, CD73 deficient (CD73KO) mice were subjected to a classical TNCB-induced CHS protocol and we found increased ear swelling reactions as compared to wild-type mice, indicating rather down-modulatory functions of CD73 in CHS reactions.

To accurately assess the tolerogenic functions of CD73 expression by smDC in CHS reactions, we performed a tolerance model whereby application of DNTB renders animals tolerant to subsequent sensitization with DNFB. Here we show that both, the tolerogen DNTB as well as the sensitizer DNFB induced migration of similar DC subsets from skin to draining lymph nodes. But animals treated with DNTB showed higher frequencies of CD73+ cells, in particular in CD11b+ as well as in CD207+CD103+ smDCs subsets. Of note, CD73KO animals were resistant to tolerization by DNTB.

Thus, these data indicate a substantial role of CD73+ skin DC in regulating tolerance to haptens.

P145 (OP06/01) | Expression of the aryl hydrocarbon receptor in cutaneous langerin-positive dendritic cells is crucial for disease development in a mouse model of systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is an autoimmune disease caused by autoreactive T and B cells. The conversion of established immunotolerance into chronic inflammation can be triggered by increased CD40-CD40 ligand (CD40L) signaling on immunocompetent cells. Accordingly, in a transgenic mouse model the overexpression of CD40L in basal keratinocytes (K14-CD40L tg) resulted in a SLE-like autoimmune disease including dermatitis, nephritis and the presence of serum autoantibodies. Virtually all cell types present in the skin express the aryl hydrocarbon receptor (AhR), a sensor of environmental stimuli, like UV-light or dioxin like chemicals, both known as risk factors for SLE. In support of this, we could show that AhR is highly up regulated in lesional skin, CD8+ T cells and dendritic cells (DCs) from K14-CD40L tg mice compared to wild-type (wt) controls. To study the effects of the AhR, known to control T cell activation and Langerhans cell maturation, on CD40L-induced systemic autoimmunity, K14-CD40L tg mice were crossed to AhR-deficient animals. Surprisingly, double mutants showed a delayed onset of disease and a reduced severity of dermatitis compared to tg controls, accompanied by a rescued renal function and the absence of serum autoantibodies. Moreover, double mutants exhibited a decreased migration of langerin+ DCs to regional lymph nodes and a down-regulated capacity to induce CD8+ effector T cell priming. This resulted in reduced levels of follicular helper T cells as well as autoreactive CD8+ T cells in draining lymph nodes and lesional skin and finally, the amelioration of dermatitis. Interestingly, mirroring the effect of an AhR-knockout, K14-CD40L tg mice fed with a diet devoid of dietary AhR ligands were found to be protected against the disease development. CD8+ T cells are crucial for the onset of disease since adoptive transfer of CD8+ T cells from autoimmune-prone K14- CD40L tg mice induced disease in wt recipients. Notably, while a cell-specific knockout of AhR in T cells did not confer protection, loss of AhR in langerin+ DCs was sufficient to delay the onset of disease. Ablation of AhR in langerin+ DCs resulted in reduced frequencies of langerin+ DCs, T-follicular helper cells and plasmablasts in regional lymph nodes and the absence of serum autoantibodies, indicating a critical role of AhR in this DC subset for the control of self-tolerance. Together, our data suggest that AhR expression in langerin+ DCs is crucial for the pathophysiology and progression of CD40L-induced systemic autoimmunity.

P146 | IgG-opsonized tumor cells attenuate the positive feed-back loop between human pro-inflammatory dendritic cells and natural killer cells

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The concept of cancer immunosurveillance represented by a complex and multilayered interaction between immune cells and malignant tumor cells has provided the basis for very potent immunotherapy treatments of cancer. NK cells and 6-sulfo LacNAc dendritic cells (slanDCs) are innate immune cells both capable of initiating anti-tumor responses based on the secretion of pro inflammatory cytokines and direct tumor cell lysis. The crosstalk between slanDCs and NK cells was reported to be driven by a positive feedback loop of IL-12 provided by TLR-activated slanDCs and IFN-γ provided by NK cells, thereby augmenting NK cytotoxicity. In addition, expression of Fcγ receptor (FcγR) III (CD16) on both cell types enables cooperative recognition of antibody opsonized tumor cells and tumor cell lysis by antibody-dependent cell-mediated cytotoxicity (ADCC). Here, we address the question whether FcγR triggering affects the positive feedback loop of the TLR stimulated NK/ slanDC crosstalk in the context of anti-tumor responses.

To assess the impact of FcγR-mediated ITAM signaling in combination with TLR signaling, we used plate-bound Intravenous Immunoglobulin (IVIg) to mimic complexed IgG and trigger FcγR signaling in NK cells and slanDCs. FcγR triggering in NK cells resulted in increased expression of activation markers and IFN-γ production, which could further be augmented by the TLR 7,8 ligand R848. Interestingly, R848 stimulated slanDCs were profoundly inhibited by additional FcγR signaling in their ability to produce IL-12 and TNF-α, even though non-stimulated slanDCs responded to FcγR cross-linking alone with increased TNF-α production. As a result of this IL-12 inhibition, also

NK/slanDC co-cultures showed reduced IFN-γ levels with complexed IgG, suggesting an attenuation of the positive effects of the IL-12/IFN-γ feedback loop. To address the therapeutical relevance of this observation, we opsonized several tumor cell lines with therapeutic antibodies and incubated them with slanDCs and NK cells. Again, additional FcγR triggering after opsonization decreased IL-12 in R848 stimulated slanDCs compared to non-opsonized tumor cells, whereas NK cells responded with increased IFN-γ levels. In a R848-treated NK/slanDC co-culture, opsonization of tumor cells resulted in a decreased IFN-γ expression, supporting the hypothesis that the presence of an FcγR stimulus potentially acts via slanDCs to regulate NK cells.

Taken together, our data suggests that TLR-stimulated slanDCs are negatively regulated by FcγR signaling. Also, this influences the IL-12 dependent positive feedback loop between slanDCs and NK cells and thereby has potential implications for immunotherapy approaches targeting those cell types.

P147 | 4-1BB overexpression in basal keratinocytes induces the disruption of the eye ′s immune privilege

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4-1BB (also called CD137 and TNFRS9) belongs to the tumor necrosis factor receptor superfamily (TNFRS), and has a crucial role as a costimulatory molecule in a variety of immune processes. While absent on resting T cells, the 4-1BB expression is upregulated upon T cell activation. The signal triggered by 4-1BB- 4-1BB ligand (4-1BBL) is involved in the regulation of anti-tumoral immunity or the progression of autoimmunity. The overall effect is an enhanced inflammatory response. A mouse model with overexpression of 4-1BB under the control of keratin-14 (K-14) promoter was generated. These animals are characterized by 4-1BB overexpression in basal keratinocytes. Beginning at the age of three months, K-14-4-1BB tg mice develop a pruritus-like skin disease characterized by inflammatory skin lesions at the ears, snout, and neck, and an increased scratch behavior. Surprisingly, besides severe pruritus, K14-4-1BB tg mice spontaneously develop uveitis and anterior cataract at the age of 3 weeks, which is associated with the infiltration of immune cells into the eye, finally resulting in blindness. In this study the cellular and molecular mechanisms underlying 4-1BB-mediated uveitis and anterior cataract were characterized in detail and moreover, the role of 4-1BB signaling for the maintenance of the immune privilege in the eye was analyzed. At the age of 3 weeks 100% of tg mice showed phenotypic alterations of the anterior chamber as well as an opacification of the lens. Moreover, the transgenic mice′s anterior chamber exhibited a progressive worsening with the time of the alteration. Furthermore, immunofluorescent microscopy as well as qPCR analysis and FACS were used to characterize the cell infiltrate, revealing an infiltration of TNF-α expressing Gr-1+Ly-6G+ neutrophils, MHCII+F4/80+ macrophages and MHCII+CD11c+ dendritic cells in the anterior chamber of 4-1BB tg mice whereas immune cell infiltrations were completely absent in eyes from wt controls. Of note, immune cells were preferentially present in areas with high 4-1BB expression such as the ciliary body. Notably, these effects were not mediated by UV light since temporary tarsorrhaphy before disease development did not ameliorate 4-1BB-mediated uveitis or anterior cataract. Together, these data strongly suggest that 4-1BB signaling in keratinocytes is critically involved in the development of uveitis and anterior cataract and might play an important role for maintaining the immune privilege of the eye.

P148 | Early changes in keratinocytes and immune cells under antipsoriatic therapies

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Psoriasis is a chronic inflammatory skin disease characterized by aberrant keratinocyte proliferation and inflammation. The disease is associated with the expression of innate cytokines and adaptive immune responses orchestrated by interleukin (IL-)17-producing CD4+ T cells (Th17 cells). In line with this, modern targeted therapies with biologics neutralizing innate cytokines like TNF or cytokines involved in the Th17 pathway (IL-23/IL-17) are highly effective in psoriasis. Alternatively, most patients respond rapidly to topical treatment with traditional therapeutics like anthralin. In these patients psoriatic plaques clear within 2 to 3 weeks. While modern biologics have selective mode of actions, the psoriasis-improving effects of anthralin on keratinocytes and immune cells are not fully understood. Here we studied the early effects of anthralin on psoriatic skin, especially on keratinocyte biology and immune cells.

We performed histological stainings of lesional psoriatic skin before and during early phase of treatment and found a significant reduction in epidermal thickness and keratinocyte proliferation as determined by Ki67 staining. We also studied keratinocyte differentiation in psoriatic skin samples by immunofluorescence staining of keratins 5, 10 and 16 and found significant changes. Immunofluorescence stainings of skin sections and quantitative PCR analysis showed a reduction of infiltrating Th17 cells and associated cytokines. Some of the cytokines implicated in psoriasis pathogenesis were differently regulated by anthralin than by treatment with neutralizing anti-cytokine antibodies.

P149 | Topical PDE4i treatment of psoriatic lesions leads to amelioration of inflammatory markers

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Psoriasis is a common, chronic, relapsing/remitting, inflammatory skin disease with a prevalence of 2%-3%. Hyperproliferation of keratinocytes, skin infiltration of immune cells, as well as secretion of pro-inflammatory cytokines like IL-17 are considered as hallmarks of the disease.

Phosphodiesterases, and first of all the isoenzyme 4, are established therapeutical targets in inflammatory diseases. However, several side effects including nausea have been observed upon systemic application. In this study, topical application of PDE4 inhibitors (PDE4i) on inflamed psoriatic skin lesions was performed and expression of inflammatory markers was investigated.

Seven patients with chronic plaque psoriasis were subjected to skin punch biopsy before and after treatment with roflumilast 0.5% cream, TAK-084 5% cream, and vehicle cream, respectively. Inflammatory biomarkers were assessed by immunohistology and transcriptome analysis. We detected a significantly decreased keratinocyte proliferation by means of reduced numbers of Ki-67+ cells in skin biopsies treated with PDE4i when compared to vehicle cream. Further on, treatment with the PDE4i roflumilast led to a significant decrease in CD4+ cell numbers and to a significant induction of cytokeratin14 compared to vehicle cream.

Applying transcriptome analysis, we detected substantial decreases in mRNA levels of psoriatic marker genes, including human beta-defensin 2, members of the S100-family, IL-19, and the skin differentiation markers late cornified envelope 3A, hornerin, and cytokeratin 16. Roflumilast did generally show more pronounced effects compared to samples treated with TAK-084.

In conclusion, the observed reduction of markers for hyperproliferation and inflammation suggests that topical PDE4i may represent a promising treatment option in psoriasis and other inflammatory skin diseases.

P150 | Lipocalin-2 is expressed by activated granulocytes and keratinocytes in affected skin and serves as a novel blood biomarker of disease activity in acne inversa

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Background: Acne inversa (AI)/hidradenitis suppurativa is a chronic inflammatory disease characterized by painful axillary, inguinal, and perianal skin lesions with deep-seated inflamed nodules, abscesses, and fistulae.

Objectives: As the pathogenesis of AI is poorly understood and biomarkers reflecting the inflammatory process are missing, this study intended to shed light on these issues.

Methods: Epidemiologic and anamnestic data of AI patients and controls were collected. Assessment of blood, skin, and cultures of primary cells was carried out by ELISA, qRT-PCR, and immunohistochemistry.

Results: Among 35 mediators quantified in blood of AI patients, lipocalin (LCN)-2 appeared as one of the most significantly upregulated parameters compared to healthy participants. Strongly elevated LCN2 expression was also present in AI lesions, with keratinocytes and, in particular, granulocytes being sources of this expression. Among AI relevant cytokines like IL-1β, IL-17, IL-22, and IL-36, TNF-α was the only mediator which induced the LCN2 secretion in granulocytes in vitro. Furthermore, TNF-α upregulated LCN2 production in keratinocytes, and a positive relationship between systemic TNF-α and LCN2 levels was evident for AI. IL-17 strengthened TNF-α-induced LCN2 production in keratinocytes. LCN2 is a soluble glycoprotein exerting a broad range of functions that include metabolic control and induction of inflammatory pain – aspects that are of high relevance for AI. Importantly, LCN2 also acts as chemoattractant for neutrophilic granulocytes and promotes granulocyte adhesion and extravasation. Accordingly, we disclosed that LCN2 blood levels correlated with AI disease severity, but not with disease duration, age, sex, BMI or smoking habit. More detailed analyses revealed a link with the number of skin regions containing nodules and fistulae, but not scars.

Conclusions: Our study suggests LCN2 as a blood biomarker for objective assessment of inflammatory activity in AI and recommend early anti-TNF-α treatment in patients with high LCN2 levels. Additionally, it suggests the existence of a vicious circle comprising TNF-α, neutrophilic granulocytes, and LCN2, that contributes to the recurrent skin neutrophil infiltration resulting in AI-typical purulent exudate.

P151 | Serological investigation on cellular activation levels in patients with bullous pemphigoid

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As the most common member of the pemphigoid diseases bullous pemphigoid (BP) has gained attention in research due to its rising incidence over the last ten years with elderly people aged above 70 being most affected. Autoantibodies directed against structural proteins of the dermal-epidermal junction lead to tense blisters and erosions on skin as well as on mucous membranes. Although the hemidesmosomal

target antigens BP180 and BP230 have been identified and treatment options such as corticosteroids are available, the knowledge about immune cell activation in BP still relies mainly on research results gained from mouse models.

In our project it was our goal to demonstrate and compare the diverse activation of different human immune cells in patients with bullous pemphigoid in correlation to BP180 and BP230 antigen serum levels. The activation status of neutrophil granulocytes was analyzed by detection of Myelinperoxidase (MPO), S100A12 and soluble CD62L (L-Selectin) in the serum of BP patients vs normal controls. For the activation status of eosinophil granulocytes we analyzed serum eosinophil cationic protein (ECP), for mast cells the mast cell tryptase (MCT) and for T cell activation soluble CD4 and soluble Interleukin-2 receptor (sIL-2R). We analyzed B cell activation by measuring serum levels of IgD and thrombocyte activation by measuring soluble CD62P (P-Selectin). To analyze monocyte activation we detected serum neopterin levels. Sera from 25 age-matched BP patients were compared to 65 healthy age-matched donors using protein specific ELISAs. Furthermore the serum levels of the above mentioned proteins of ten BP patients at the time of acute BP disease were compared to protein serum levels from the same ten BP patients after therapy when their BP180 levels had been tested negative.

Our results show that neutrophils seem to be highly activated during BP in humans. Serum levels of MPO presented a higher concentration in BP patients than in the controls. Furthermore, S100A12 protein concentration was significantly higher in the BP patients, highlighting the immunopathogenesis of this cell type in BP, whereas sL-Selectin levels were similar in both groups. ECP concentration was significantly higher in the patient group, thus eosinophil granulocytes seem to be activated during active BP. The activation of mast cells, measured by MCT serum levels, did not show a significant difference between the two groups. T cell activation was proven by an increase in sCD4 concentration in the BP patients. Surprisingly sIL-2R levels and the concentration of IgD were similar in both groups. In contrast platelets seem to play a role in the immune reaction during BP: sP-Selectin was significantly higher in the patient group. The neopterin serum levels instead were similar in both patients and controls.

The BP treatment group did not show strong differences in serum levels of both groups for neutrophils (MPO, S100A12 and L-Selectin) and also not for eosinophil granulocytes (ECP). But a significant difference in activation of mast cells (MCT) could be seen for the BP180 positive patient group, whereas this cannot be stated for T cell activation. In addition, total neopterin serum levels showed a significantly higher concentration and also the thrombocyte derived cell adhesion molecule soluble P-Selectin levels were significantly higher in the BP180 positive patients.

The results of this study show immune cell activation in the case of neutrophils (S100A12), eosinophils (ECP) and mast cells (MCT) as well as in T cells (sCD4) and thrombocytes (P-Selectin). This investigation expands existing knowledge about the immune cell types which play an essential and critical role in autoimmune bullous pemphigoid.

P152 | Identification of keratinocyte differentiation-associated IL-1 family members

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Members of the interleukin (IL)-1 family of cytokines, including the prototypical representatives IL-1α and IL-1β, are central regulators of inflammatory reactions. Here, we investigated the evolutionary history and the expression of IL1 family genes during terminal differentiation of human epidermal keratinocytes. We identify orthologs of IL1 and IL36 in reptiles whereas IL37 and IL38 are specific to mammals. Whales and dolphins, in which the epidermal differentiation program has degenerated in association with the acquisition of a fully aquatic lifestyle, have lost functional IL36A, IL36B, IL37 and IL38 genes. When human epidermal keratinocytes were stimulated to undergo differentiation in vitro the transcription of IL36A, IL36B, IL37 and IL38 genes was upregulated. Western blot analysis showed a strong increase in IL37 protein abundance in differentiating human keratinocytes, and immunohistochemistry demonstrated expression of IL37 in the granular layer of normal human epidermis. These results suggest that the expression of IL37 and three other IL1 family members in differentiated keratinocytes contributes to the homeostasis of normal epidermis.

P153 | Xenoreactivity to rabbit IgGs contributes to pathogenesis of experimental epidermolysis acquisita in mice

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The incidence of autoimmune diseases is increasing worldwide, which leads to the need to develop new therapeutic options. To identify new therapeutic strategies several mouse models have been established during the past years in which antibody-mediated autoimmune diseases are imitated by passive transfer of autoantibodies specific for mouse proteins but generated in a different species; mainly in rabbits. This approach is successfully used in models such as rheumatoid arthritis, bullous pemphigoid or epidermolysis bullosa acquisita (EBA). However, foreign antigens are recognized by pattern recognition receptors such as Toll-like, NOD-like receptors or by natural antibodies or complement and can induce an activation of immune cells. Data about the effects of xenoreactivity on disease development after passively transferred IgGs are lacking.

Here, we utilized the experimental model of the autoimmune skin blistering disease EBA to evaluate the T and B cell responses in the draining lymph nodes of C57BL/6 mice after injection of rabbit

IgGs that are directed against mouse collagen type 7 (anti-mCol7), a constituent of anchoring fibrils of the dermal-epidermal junction. Our data show that rabbit anti-mCol7 IgGs induced T cell proliferation and the expression of the T helper cytokines IFN gamma and IL4 in lymph nodes and skin lesions and subsequently, the formation of germinal centers. Interestingly, treatment with the T-cell emigration blocker FTY720 decreased the expression of IFN gamma in skin lesions indicating that activated rabbit IgG-specific T cells migrated into the lesions and contributed to the cytokine milieu in affected skins. In addition, we observed that mice produced IgGs directed against anti-mCol7 rabbit IgGs that bound to the dermal epidermal junction (DEJ) of the skin. The prevention of anti-rabbit IgG production by utilizing B-cell deficient or CD154-deficient C57BL/6 mice lead to a decreased disease score in EBA mice. In conclusion, our data clearly demonstrate that xenoreactivity to rabbit IgGs modulates development of EBA: First, T cells change the cytokine milieu towards Th1 in EBA skin lesion. Second, mouse-derived anti-rabbit IgGs appear as auto-antigen by binding to the rabbit IgG at the DEJ and significantly worsen the clinical picture of EBA especially at later time points. This data demonstrate that the results obtained in experimental mouse models that were induced by passive transfer of xenogeneic IgGs might be skewed by xenoreactive responses of the recipient. Whereas these mouse models are well suited to study the early effector phases the impact of xenoreactivity should be carefully considered by investigating later time points.

P154 | Dimethyl fumarate modulates Neutrophil extracellular trap formation in an L-glutathione and superoxide dependent manner

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Neutrophil extracellular trap (NET) formation is a recently discovered mechanism by which neutrophils release a lattice of chromatin strands decorated with antimicrobial peptides into the extracellular space. The main function of NETs is to trap and possibly kill pathogens in cutaneous and systemic infections. However, they also function as danger-associated molecular patterns, and create a pro-inflammatory environment in which self-antigens are amenable to the immune system. Neutrophils are a histologic hallmark of psoriasis and among the first cells to infiltrate nascent plaques. Furthermore, they were reported to release LL-37/ DNA complexes and IL-17 via NET formation in psoriatic skin. In the present study, we found that neutrophils from psoriasis patients on routine treatment with a fumaric acid ester (FAE) formulation formed significantly less NETs than neutrophils from healthy donors and psoriasis patients without systemic treatment combined. Pre-treatment of healthy donor neutrophils with the FAE dimethyl fumarate (DMF), a small lipophilic molecule, resulted in a consistent and dose dependent inhibitory effect on NET formation. This effect was L-glutathione dependent and involved the reduction of reactive oxygen species production, a key event in NET formation. In contrast, G-protein-dependent signaling or translation of new proteins were not necessary. The effect of DMF was stimulus specific, as NET formation to phorbol 12-myristate 13-acetate, but not to platelet activating factor and ionomycin, was reduced. In conclusion, we report DMF as a potent modulator of PMN function, in particular NET formation. These mechanisms may contribute to the beneficial effects of FAE treatment in inflammatory diseases.

P155 | Targeting tumor-associated macrophages in a human melanoma model using siRNA and small molecules encapsulated in nanoparticles to achieve an antitumor response

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Introduction: Tumor cells escape the patient's immune system by inducing immune suppression in the tumor microenvironment. Tumor associated macrophages (TAM) are major players of the tumor microenvironment and have been shown to promote tumor growth by inducing neoangiogenesis, supporting metastasis and rendering tumor infiltrating lymphocytes (TIL) suppressive or apoptotic.

Objectives: By disrupting the signal pathways responsible for TAM phenotype via siRNA mediated gene knockdown targeting receptors (*IL-4R & CSFR1*) and/or downstream transcription factors (*STAT6*, *IRF4 & NOR1*), we try to reprogram tolerance inducing TAM to classically activated immunostimulatory M1 macrophages. To avoid degradation and unspecific cellular uptake siRNA is bound to or encapsulated in nano-sized carriers.

In a second approach we use dendritic mesoporous silica nanoparticles and liposomes as drug carriers for metformin, BLZ945, sorafenib and tasquinimod which have been shown to influence the phenotype of macrophages.

Methods: In vitro culture of human monocyte-derived macrophages and THP-1 cells. Analysis of the phenotype via flow cytometry (surface markers and intracellular staining), qPCR (gene expression), microscopy (morphology and nanoparticle uptake) and cytometric bead assay (secreted cytokines). Viability assays. Generation of tumor conditioned media from human melanoma cell lines.

Cationic polymer- and dextran-based nanoparticles as siRNA-carriers. Dendritic mesoporous silica nanoparticles and liposomes as drug carriers.

Human Melanoma model in the humanized mouse with subcutaneous and hepatic tumors.

Results: To screen potential siRNA targets for their ability to repolarize M2 macrophages we have successfully transfected human macrophages by inducing a knockdown for *IL4R*, *NR4A3*, *STAT6* and *PPARG* which altered the phenotype of human macrophages in vitro.

Acid degradable cationic dextran particles, which are able to efficiently encapsulate siRNA and have a size range of 100 to 150 nm, already proved to be a promising candidate because of low toxicity and high uptake rates in monocytes and macrophages without influencing the phenotype. In wild-type mice, nanoparticles accumulated preferentially in the liver where they showed high uptake rates in liver macrophages (70%-80%).

Freshly isolated human monocytes responded to BLZ945 treatment with a higher toxicity rate and downregulation of CD206 and CD14 compared to monocytes which received only M-CSF.

Conclusion: In summary, the use of nanoparticles as drug and siRNA delivery systems targeting TAMs promises enormous potential to modulate immune tolerance towards tumors. siRNA mediated gene knockdown alters the phenotype of IL-4 polarized macrophages. We will consequently validate those in vitro effects in our in vivo tumor model.

P156 | Investigating immunosuppressive regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC) in checkpoint inhibitor therapy of malignant melanoma

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Immunotherapy targeting the advanced stages of malignant melanoma intends to enhance the chances of long-term survival. Checkpoint inhibitors like the monoclonal antibody anti-CTLA-4 (ipilimumab) and the anti-programmed death 1 (PD-1) inhibitors pembrolizumab and nivolumab aim to overcome immune tolerance.

Unfortunately, reactivation of T effector cells is accounted for as an often delayed effect in a part of treated patients.

Importantly, there are currently no reliable biomarkers available to early identify and predict responders. Whereas immunomonitoring revealed high frequencies of regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC) in melanoma patients. The predictive value of both populations remains uncertain.

In the present study, we aimed to identify novel cellular and soluble biomarkers to single out responders to immune checkpoint inhibitors at the early stages of therapy.

Therefore we analyzed plasma and blood from 40 melanoma patients treated with ipilimumab, nivolumab or pembrolizumab. By comparing protein levels in responders and non-responders we identified a panel of potential soluble biomarkers using a quantitative proteomic analysis of plasma samples. Two candidates of this training set could already be validated in additional assays such as ELISA.

To correlate our novel biomarkers to previous data on MDSC and Treg, immunomonitoring included analysis of frequencies and functionality of Treg and MDSC subpopulations. Preliminary data displayed a decrease in immunosuppressive MDSC in responders.

To conclude, we identified novel potential biomarkers differentiating responders and non-responders to checkpoint inhibitor therapy. Moreover, we found that immunosuppressive MDSC decrease stronger in responders to ipilimumab therapy thereby favouring an immune response against the tumor.

P157 | CAR/TCR-transfected γ/δ T cells for immunotherapy of melanoma

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T cells transfected with tumor antigen-specific chimeric antigen receptors (CARs) or T-cell receptors (TCRs) have been successfully used for adoptive therapy of cancer. However, if conventional T cells are used, the endogenous α/β TCRs may cause autoimmunity due to activation of dormant self-reactive T cells or by mispairing with the introduced α/β TCR chains. To counteract this problem, it would be advantageous to equip γ/δ T cells, which represent a potent subpopulation of peripheral T cells with known endogenous anti-tumor activity, with a tumor antigen-specific α/β TCR or CAR. This may not only increase safety of adoptive T-cell therapy, but may also result in an effective strategy: In addition to the cytotoxicity induced by the exogenous receptor, the anti-tumor activity of these cells may be enhanced via the endogenous γ/δ TCRs. The aim of this study was to establish a GMP-compliant protocol to expand and transfect γ/δ T cells using electroporation of receptor-encoding mRNA.

PBMCs from healthy donors were stimulated using zoledronic acid or anti-CD3 antibody (OKT3) to expand γ/δ T cells and bulk T cells, respectively. Additionally, CD8+ T cells and γ/δ T cells were MACS-isolated from PBMCs and expanded with OKT3. After 10-11 days of expansion, these four populations were electroporated with RNA encoding a gp100/HLA-A2-specific TCR or a CAR specific for melanoma-associated chondroitin sulfate proteoglycan (MCSP). Receptor expression, antigen-specific activation and cytokine secretion, specific cytotoxicity, and killing of MHC-deficient Daudi cells were analyzed following transfection of T cells and stimulation with target cell lines.

Using zoledronic-acid, in average 6 million γ/δ T cells with a purity of 85% could be generated from one million PBMCs. MACS-isolation

and OKT3-mediated expansion of γ/δ T cells yielded approximately ten times less cells. OKT3-expanded and CD8+ MACS-isolated conventional T cells behaved correspondingly similar. All employed T cells were efficiently transfected with the TCR or the CAR. Upon respective stimulation, γ/δ T cells produced IFN-γ and TNF, but little IL-2 and the zoledronic acid-expanded T cells exceeded MACS-γ/δ T cells in antigen-specific cytokine secretion. While the cytokine production of γ/δ T cells was in general lower than that of conventional T cells, specific cytotoxicity against melanoma cell lines was similar. In contrast to OKT-3-expanded and MACS-CD8+ T cells, mock-electroporated γ/δ T cells also lysed tumor cells, which reflect the γ/δ T cell-intrinsic antitumor activity. After RNA transfection, the γ/δ T cells still responded to stimulation of their endogenous TCR with up-regulation of CD25 and CD69 and with specific secretion of IFN-γ and TNF. In addition, they were still able to kill MHC-deficient Daudi cells.

In conclusion, we generated a GMP-compliant protocol for the expansion of γ/δ T cells and their subsequent transient transfection with tumor-specific TCRs or CARs using zoledronic-acid in conjunction with mRNA-electroporation. While functionality of engineered γ/δ T cells was similar to conventional engineered T cells, the generation of engineered γ/δ T cells represents a safer method and thus can be used as an effective tool in the immunotherapy of melanoma.

P158 | The imiquimod model as a model for psoriasis and/or eczema in humans?

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Many effective therapies have been developed in the past decades for both eczema and psoriasis, yet both diseases are still underdiagnosed and undertreated. Due to their complexity and heterogeneity not sufficiently acknowledged in most studies, the pathogenesis of psoriasis and eczema is not fully understood and novel human models would be needed to achieve a substantial scientific break-through. In contrast to eczema, for which at least the subtypes acute contact dermatitis and irritant dermatitis can be directly induced in humans by local application of the allergen or irritant, there is no proper human model for psoriasis. Observational studies in humans reported exacerbation or new onset of psoriasis in patients who had been treated with imiquimod cream for skin cancer. This phenomenon has been translated into one of the most commonly used murine models for psoriasis in which mice daily treated with imiquimod cream develop a psoriasis-like dermatitis. The aim of this project was to elucidate which aspects of inflammation typically seen in psoriasis and/or eczema would be reflected by the human imiquimod model and if – as application of imiquimod cream may result in a standardized and thus reproducible robust iatrogenically induced skin inflammation – at least some of the complex pathways of psoriasis and/or eczema could be studied and altered within this model. We found that in a cohort of healthy volunteers and patients suffering from eczema and/or psoriasis (n=14) application of imiquimod cream on non-lesional skin lead to a homogenous eczema-like reaction on clinical and histopathological level independent of the genetic background. Hallmarks of psoriasis such as microabscess, parakeratosis and hypogranulosis were absent, but instead the both for psoriasis and eczema atypical finding of a deep pseudolymphoma-like lymphocytic infiltrate was made. To gain further insights on molecular level, whole-genome expression arrays of imiquimod treated skin (IMQ) samples were performed and compared to an independent group of psoriasis (n=24), chronic eczema (n=14) and acute contact dermatitis (ACD, n=10). Analysis of top regulated genes as well as gene enrichment analysis revealed the highest number of genes commonly regulated when comparing ACD, but not chronic eczema or psoriasis, to IMQ. Both IMQ and ACD were characterized by inflammasome activation including the NF-B/interferon regulatory factor 1 (IRF-1) signaling pathway. Moreover, when comparing IMQ to psoriasis, we found upregulation of the IL-23 pathway, a key pathway in psoriasis, also in IMQ. Taken together, the human imiquimod model has the potential to be an alternative to murine models for explicit (interventional) questions to be studied in patients. Ongoing research will reveal the cellular and molecular reasons for the incomplete psoriatic phenotype in human as compared to murine skin.

P159 (OP03/04) | Towards the analysis of the paired TCR alpha- and beta-chains of single alopecia areata-specific human CD8+ T-cells

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The most common autoimmune hair loss disorder, alopecia areata (AA), is an organ-restricted, CD8+ T-cell-dependent autoimmune disease that attacks hair follicles (HF) which have lost their immune privilege. However, the MHC class I-presented (auto-)antigen(s), that are likely expressed in the proximal hair bulb of lesional HFs in AA patients, and the T-cell receptor (TCR) repertoire of the primary autoreactive CD8+ T-cells remain unknown. Therefore, all currently available AA therapies are purely symptomatic, rather than curative. To address this challenge, we have adopted the strategy to analyze the TCR repertoire by systematically screening the TCR alpha- and beta-chains of intra- and perilesional CD8+ T-cells as a basis for subsequently identifying the pathogenic (auto-)antigen(s). Therefore, we have isolated disease-specific, intra- and perilesional CD8+ T-cells from AA skin by laser microdissection in order to determine their TCR

clonotype in situ. This is the only method which allows one to distinguish peri- from intra-lesional infiltrating CD8+ T cells while simultaneously identifying their paired alpha- and beta-chains.

Up to now, we have been able to characterize several TCR beta-chains of such autoreactive CD8+ T-cells, namely Vbeta7, 12, and 27, and the corresponding alpha-chains, specifically Valpha4, 10, and 13 (including CDR regions) from the skin of AA patients, whose HLA type was characterized. In selected, but not all, investigated AA patients, these transcriptional data were confirmed by immunohistology, using the few commercially available beta-chain antibodies, which detected corresponding beta-chain TCR proteins. So far, our protein level in situ results point to Vbeta12 as the most widely expressed beta chain in AA lesions, followed by Vbeta13. However, since the most expanded CD8+ T-cell TCR clonotypes vary greatly from patient to patient, we are currently expanding the number of CD8+ T-cells investigated in situ from each examined AA patient. Once disease-specific TCRs have been identified, even if the corresponding (auto-)antigens have not been characterized yet, this can serve as a basis for TCR-specific lymphocyte elimination immunotherapy in AA, and may also provide prognostic biomarkers.

P160 | Analysis of autoreactive desmoglein 3-specific B cells in patients with pemphigus vulgaris

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Pemphigus vulgaris (PV) is an autoimmune blistering disease caused by autoantibodies (auto-ab) mainly against the desmosomal cadherins desmoglein (Dsg) 3 and Dsg1 leading to painful blisters and erosions of the skin and mucous membranes. While the pathogenic role of Dsg3-reactive auto-ab in PV is extensively investigated, comprehensive studies analyzing pathogenic Dsg3-specific B cells in PV are lacking. In this cross-sectional study we established a flow cytometry-based method to detect Dsg3-specific B cells in peripheral blood of PV patients by using recombinant human Dsg3 protein labeled with fluorescent dye Alexa Flour (AF) 647. First, specificity and sensitivity of this technique was evaluated using monoclonal mouse B cell hybridoma (BCH) cells that were specific for human Dsg3. Pilot experiments demonstrated high specificity of this approach since binding of Dsg3-AF647 was limited to Dsg3-specific BCH in comparison to control BCH with an unrelated specificity. In addition, fluorescently labeled recombinant human collagen7 protein was used as negative control. Dsg3-AF647 was used to analyze Dsg3-specific B cell compartments (including CD19+CD27- naïve B cells and CD19+CD27+ Memory B cells) in peripheral blood of clinically-well defined PV patients (n=14). Here, we show that Dsg3-specific B cells are increased in PV patients compared to healthy controls (HC; n=10). Interestingly, Dsg3-specific B cells were also detected in HC at low frequencies suggesting the presence of non-pathogenic autoreactive B cells in healthy subjects, as well. Comparing the different clinical activities of PV patients we observed that the highest numbers of Dsg3-specific memory B cells were present in PV patients in clinical remission on minimal therapy whereas in PV patients receiving high immunosuppressive treatment, memory Dsg3-specific B cells were markedly reduced. Our results show that systemic immunosuppressive treatment exerts a decreasing effect on the frequency of Dsg3-specific B cells in peripheral blood of PV patients. However, the presence Dsg3-specific memory B cells in remitting PV patients points towards an ongoing reconstitution of autoreactive B cells that could cause a relapse in the future. Further monitoring of Dsg3-specific B cells in remitting PV patients can be used as a helpful tool for therapeutic interventions preventing future disease relapse.

P161 (OP06/03) | Characterization of multiple B cell subsets in peripheral blood and skin biopsies of psoriasis patients identifies a correlation of plasma and regulatory B cells and disease severity

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Imbalances of T cell subsets have been demonstrated as hallmarks of disease-specific inflammation in psoriasis. However, the role of B cells as important counterparts of T cell function remains poorly investigated. In a cohort study, we analysed a broad set of B cell subsets and immunoglobulins and correlated their distribution in peripheral blood with disease severity in psoriasis patients. Then we analysed if observed changes could be validated in the respective psoriatic skin samples. Finally, in a small prospective study cohort, we investigated the impact of systemic treatment on cellular and humoral parameters.

Surface staining and flow cytometry was performed on leucocytes from whole blood of 55 psoriasis patients and 20 individuals without history of skin disease. The severity of psoriasis was determined by Psoriasis Area and Severity Index (PASI) and patients were classified as PASI low (<5) or PASI high (>10). B cell subsets were defined by their expression of CD24, CD38, CD138 and CD27. The humoral immunological profile was complemented by serum parameters including immunoglobulins. We found a significant increase of plasma cells (CD19+ CD38++ CD138+) accompanied by increased IgA serum levels in patients with higher severity scores (PASI high) as compared to patients of the PASI low group. Moreover, frequencies of CD138+ regulatory B cells (CD19+ CD24high CD38high) were upregulated in psoriasis patients compared to healthy donors. Immunofluorescent

staining of psoriatic skin biopsies (n=18) for IL10+ regulatory B cell revealed a trend of increasing numbers of regulatory B cells with increasing disease severity. Another immunofluorescent staining of IgA will show whether cutaneous IgA deposits also correlate with disease severity. For 10 of the initially untreated patients a second blood analysis after successful treatment with a systemic medication was performed. Ongoing biocomputational analysis will validate a correlation of the observed parameters with disease severity in this prospective cohort.

These data suggest a contribution of certain B cell subsets to the severity of psoriasis with increased frequencies of regulatory B cells representing a possible compensatory mechanism to increased frequencies of plasma cells and IgA serum levels observed in psoriasis patients.

P162 | Regulation of IL-33 expression in epithelial cells and immune cells

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Key mediators of innate immunity during skin inflammation are cytokines of the interleukin-1 (IL-1) family. While IL-1β and IL-18 are well characterized and are activated via inflammasome activation, the activation of IL-33 is not fully understood. Recent data suggest that IL-33 acts as an alarm or danger signal and is released after cell damage. Besides binding to its specific receptor ST2, IL-33 can be present in the nucleus and in the cytosol.

Here, we aim to elucidate the induction of IL-33 and its receptor ST2 compared to the other IL-1 family members IL-1α and IL-1β in different cell types and studied the cellular localization of the alarmin IL-33. We analyzed human and murine myeloid cells and epithelial cells and tested the expression of IL-33 mRNA and protein after stimulation of different pattern recognition receptors. The cellular localization of IL-33 was identified by Western blotting of nuclear and cytosolic cell extracts and by immunofluorescence.

Interestingly, we found very different expression levels of IL-33 in certain myeloid cells and tissue-resident cells in both human and mice. High baseline levels of IL-33 were even present in cells that do not carry an inflammasome and are not able to activate inflammatory caspases. In analogy to IL-1α and IL-1β, IL-33 can be induced in myeloid cells by certain stimuli and signaling pathways. However, expression levels and kinetics of IL-33 seem to be different from IL-1α and IL-1β.

In conclusion, we demonstrate a different activation pattern of IL-1α, IL-1β and IL-33 in myeloid and non-myeloid cells. As all these innate mediators are released during cutaneous damage we hypothesize that the activation of inflammasomes or inflammatory caspases influences not only the innate immune response but also the adaptive immune response in very early stages of skin inflammation.

P163 | Signal deficiency of melanocortin-1 receptor augments extent of skin infection induced by *S. aureus*

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The melanocortin-1 receptor (MC1R) is crucially involved in skin melanin pigmentation in a variety of vertebrate species. It is a G protein-coupled receptor with 7 transmembrane domains and binds melanocortins such as alpha-melanocyte-stimulating hormone with high affinity. Despite its physiological role in skin pigmentation there is ample evidence that α-MSH has additional biological effects including immunomodulation, the latter often mediated by MC1R. However, the role of MC1R in cutaneous infection has not been investigated to the best of our knowledge. Here, we examined the impact of MC1R deficiency in a mouse model for cutaneous infection. MC1R signaling-deficient mice (Mc1re/e) and control mice (C57BL/6) were intradermally infected with 107 colony forming units of *S. aureus* (strain SH100). The extent of skin infection was monitored for 6 days. Interestingly, Mc1r e/e mice developed larger skin lesions compared with control mice. Gram staining revealed higher bacterial amounts in lesional skin of Mc1re/e mice than in control mice. In accordance with this mRNA expression of nusA and sigA, two established markers for *S. aureus*, was markedly increased in Mc1re/e mice. Surprisingly, the cutaneous levels of IL-6, IL-8, IL-1β and IL-17a were significantly lower in lesional skin of MC1R signaling-deficient mice than in control mice as determined by real-time RT-PCR analysis. In addition, mRNA expression of Ly6c, Ly6G and CD11b, markers of neutrophils, and subsets of monocytes/macrophages, was decreased in Mc1re/e mice compared with control mice. Using FACS analysis we quantified the number of macrophages in infected skin and found an increased number of macrophages in wild-type mice vs Mc1r e/e mice. In addition, increased numbers of T regulatory cells were detected in skin lesions of wild-type mice compared with MC1R signaling-deficient mice. In sum, these preliminary findings indicate that MC1R could be involved in controlling both the extent of infection and subsequent immune response of the skin to *S. aureus*.

P164 | Regulatory T cell defect cause Th1/Th2 driven skin inflammation in Scurfy mice

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Regulatory T cells (Tregs) require for their development and function the transcription factor Foxp3. Scurfy mice have a missense mutation in this gene and generate thereby only a truncated version of Foxp3. Scurfy mice are not able to generate functional Tregs and have

a defective peripheral tolerance, which leads to an expansion of autoreactive CD4+ T cells. In Scurfy mice different organs show an inflammation and Scurfy mice are able to develop any kind of autoimmune disease. Interestingly the liver, lung and skin are always affected. Scurfy mice show severe skin inflammation during the disease, but inflammation and flaking of the skin vary between different anatomical regions. To study which cells are responsible for the pathology and the inflammation in the skin, and if there are any differences between different skin parts, the inflammatory infiltrate of ear, tail, leg and back skin was isolated and different populations of immune cells were determined by FACS analysis. The highest frequencies of T cells, NK cells, neutrophils appear in the ear immune infiltrate, whereas infiltrates of leg, tail and back skin contain the same cells but in lower frequencies. B cells were not detectable in any of the infiltrates. It is already described, that in Scurfy mice a Th2 immune response occurs, which is linked with eosinophilia and high IgE levels. Therefore, we were interested in the cytokine profile of skin infiltrate of Scurfy mice during different disease stages. A Th1/Th2 mediated inflammation by detecting equal numbers of INF-γ- and IL4-producing cells in skin and sdLN could be shown by FACS analysis. IL4 production increased over the disease severity. These findings suggest that a lack of Tregs leads to a Th1/Th2 mediated skin inflammation in later stages of disease.

P165 | The IL-1β pathway is hyperactive and promotes tissue destruction and immune cell infiltration in Acne inversa

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Acne inversa (AI; also referred as Hidradenitis suppurativa) is a frequent chronic inflammatory disease characterized by painful deep skin lesions with purulent discharge leading to irreversible destruction of skin architecture. The high physical and mental burden of patients contrasts with limited knowledge of AI pathogenesis and restricted treatment options. Thus, in a translational approach using skin and blood samples from healthy controls and about 60 AI patients as well as numerous in vitro experiments, our study aimed to identify pathogenetic pathways involved in the destructive inflammation in AI. Investigating the cytokine pattern in AI lesions, we unraveled IL-1β as the most prominent cytokine, whose expression even exceeded that in psoriasis lesions. Subsequent analysis of the IL-1β-induced transcriptome in various skin cell types revealed a most regulations in dermal fibroblasts, correlating with a high cellular IL-1β receptor expression. Importantly, high degree of overlap of upregulations including molecules promoting extracellular matrix destruction, immune cell infiltration, and specific cytokines were observed in different cell types. This IL-1β signature was verified in vitro. Furthermore, the pattern of induced extracellular matrix-degrading enzymes and cytokines was IL-1β specific and not relevantly induced by other cytokines expressed in AI lesions. Importantly, it was also specifically present in AI lesions. Among cutaneous IL-1β target molecules, only IL-6 levels were clearly elevated in the blood of AI patients. Search for further blood parameters reflecting IL-1β pathway activity disclosed serum amyloid A (SAA), whose production was synergistically induced by IL-6 and IL-1β in hepatocytes. Consequently, strongly elevated blood SAA levels in AI correlated positively with disease severity and negatively with HDL-cholesterol levels, linking the IL-1β pathway to common cardiovascular comorbidity in AI. We conclude that an active IL-1β pathway in AI represents a pathogenetic cascade associated with chronic local tissue destruction and immune cell infiltration, is assessable through the blood biomarker SAA, and its inhibition is a promising strategy for the personalized treatment of AI.

P166 | Increase of the efficacy of vaccinations in immunosuppressed mice

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The response to vaccinations, respectively the levels of specific IgG, can be reduced by various means, such as immunosuppressive medication, radio- and chemotherapy, smoking, acquired or hereditary immune deficiency syndromes. Thus, the increase of the efficacy of vaccinations could be of great value, especially to these people.

Previous projects have identified two compounds of the Prestwick Chemical Library® as potential activators of the immune system. We have been able to show that the effect of both compounds is limited to B cells and does not affect T cells or polymorphonuclear cells in vitro. Furthermore, we have established a mouse model which strongly indicates that immunosuppressed mice show a reduced response to vaccinations regarding the levels of specific IgG. The mice were immunosuppressed with 2.5 mg/kg etanercept (Enbrel®), a TNF-alpha inhibitor, and received a vaccination with either ovalbumin, keyhole limpet hemocyanin (KLH) or tetanus. The most significant difference between the immunosuppressed and the control group was seen after vaccination with KLH or tetanus.

It is now our aim to show that the levels of total IgG, total and specific IgM and most importantly specific IgG can be increased by administration of one of both compounds on several days surrounding the

vaccination. First rounds of testing have delivered significant data when comparing the immunosuppressed group which received one of the compounds to the immunosuppressed control group. If our further research proves to be a success, we would like to underline the increased efficacy of the tetanus vaccination by administering tetanus toxin. Thus, not only showing that the levels of specific IgG are increased but also that the efficacy is elevated to the point that these mice are protected from tetanus toxin and that the vaccination is effective.

P167 (OP03/02) | Deficiency of IL-22 binding protein strengthens psoriatic skin inflammation

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Psoriasis is a chronic inflammatory skin disease that affects about 2% of the Caucasian population. Psoriatic skin alterations are supposed to results from chronic dysregulated activation of the cutaneous immune system that—by secreted cytokines—alter the biology of local tissue cells. The influence of cytokines on keratinocytes leads to massively thickened and scaling epidermis, to the production of chemokines enabling the recruitment of further immune cells to create a self-sustained inflammatory milieu, and to overexpression of antimicrobial proteins (AMP) that prevent infections of the disturbed psoriatic epidermis. So far, there is very limited knowledge about the regulation of cytokine's action in psoriasis. In a translational approach, our work focused on this issue. Skin and blood samples from healthy controls and psoriasis patients, in vitro experiments with human keratinocytes and reconstituted epidermis, and transgenic animals were used. We demonstrate that interleukin-22 binding protein (IL-22BP), a soluble factor known to inhibit IL-22 action, shows a lower expression in non-affected skin of psoriasis patients compared to skin of healthy controls. While in perilesional and lesional psoriatic skin IL-22 expression clearly increased (~5000- and 30 000-fold, respectively), the rise of IL-22BP expression was only moderate (~2-fold). This suggests that the deficiency of IL-22BP contributes to both the development and persistence of psoriatic inflammation. To test this hypothesis we first generated IL-22BPdeficient rats (Il22ra2−/−) and, by cutaneous imiquimod application, induced psoriasis-like skin inflammation in these animals. Compared to littermate controls, clinical and histological alterations (acanthosis, parakeratosis, microabscesses, dermal infiltration) were more profound in Il22ra2−/− rats. Moreover, enhanced expression of inflammatory cytokines (like IL-17A and TNF-alpha) and AMPs was observed in IL-22BP-deficient rats. In a second approach, we applied IL-22BP-neutralizing antibody to imiquimod-treated mice. Compared to mice injected with isotypic control antibody, skin erythema, thickening, and scaling were dramatically worsened. This was also confirmed by histological analyses. To further investigate the molecular mechanisms, we then got back to the human system. Hypothesizing that the IL-22/IL-22BP expression ratio reflects the level of free bioactive IL-22, we indeed found a positive association between IL-22/IL-22BP ratio and the expression of molecules with known pathogenetic roles in psoriasis (like IL-20, IL-36, and CXCL1) and with AMP expression (BD2). A clear correlation with the IL-22/IL-22BP ratio was also observed for IL-24. In vitro experiments demonstrated the IL-24-inducing effect of IL-22 in keratinocytes, which was further enhanced by IL-17, and the inhibition of the keratinocyte differentiation by IL-24. Addressing blood levels in psoriasis patients, the IL-22/IL-22BP protein ratio also strongly correlated with psoriasis disease severity. In summary, we demonstrate first data about the regulation of the activity of key cytokines in psoriatic inflammation.

P168 (OP03/05) | Intimate contact between innate lymphoid cells and T cells in inflamed human skin

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Innate lymphoid cells (ILCs) have recently emerged on the stage of cutaneous immune defense but their role in skin remains poorly understood. In this study, we aimed at developing a technique to assess ILCs in situ and to determine their topographical distribution in both normal and inflamed human skin.

We collected lesional skin biopsies from atopic dermatitis (AD) and psoriasis (Pso) patients (both n=13) and normal human skin (NHS) from healthy controls. After establishing immunofluorescence ILC in situ stainings, we developed an analysis approach (gating combined with manual validation) to reliably identify ILCs. Topographical mapping was obtained by automated calculations of the distances between ILCs and different cellular/structural elements of the skin.

Whereas NHS harbored a very scarce ILC population (mostly ILC1s and AHR +ILC3s), AD and Pso skin was infiltrated by clearly visible ILC subsets. We observed AD skin to contain not only ILC2s, but also a prominent AHR+ILC3 population. Conversely, we encountered almost equal proportions of ILC1s and RORC+ILC3s in Pso skin. Distance calculations revealed ILCs to essentially reside near the epidermis. When analyzing the spatial relationship between ILCs and T cells, we found a strikingly close proximity between these populations in both AD and Pso. As to the phenoytpe of the T cells in close proximity to ILCs, most of them did not belong to their respective innate "mirror" subsets (ie, ILCs expressing the same transcription factor) with the exception of Th2 cells, which made up for the majority of T cells surrounding ILC2s in AD.

ILC mapping in situ should help us to gain further insights into the crosstalk of these cells with other leukocytic and non-leukocytic cell populations in skin and to ultimately better understand their contribution to cutaneous immune defenses.

P169 | Control of expression and immune regulatory function of TIMP3 in psoriasis

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There is evidence that the extracellular matrix and fibroblasts exert active roles in shaping inflammatory responses. We here focus on TIMP3 an endogenous inhibitor for ADAM17 thereby regulating the generation of soluble TNF-α. Downregulation of TIMP3 was reported for keratinocytes of psoriatic skin lesions with restoration of TIMP3 inducing lesion regression. By immunofluorescent staining as well as by qRT-PCR we demonstrate a strong downregulation of TIMP3 in the dermis of psoriatic skin lesions and that normal human dermal fibroblasts express TIMP3 in a basal state. Importantly, exposure to cytokines relevant in the psoriatic pro-inflammatory milieu, namely IL-17 and TNF-α, resulted in the downregulation of TIMP3 mRNA in dermal fibroblasts. Cytokine mediated downregulation of TIMP3 correlated with a strong increase in mir21 expression, a microRNA reported to inhibit TIMP3, thus implicating novel epigenetic regulation mechanisms in the TIMP3-ADAM17 inflammatory loop in dermal fibroblasts. Our findings are relevant in the context of dermal inflammation in psoriasis. We focus specifically on inflammatory dermal dendritic cells that express 6-sulfo LacNAc: slan-dendritic cells. We demonstrate that slanDCs express active ADAM17 on the cell-surface. Addition of TIMP3 to slanDCs inhibited ADAM17 activation, blocked LPS-induced IL-23- and IL-12-production by 60-70% and attenuated the Th17/Th1 programming by slanDCs. Identical results were obtained with a highly specific ADAM17-blocking antibody D1A12. We propose protease inhibitor TIMP3 to have a strong regulatory potential over dermal inflammatory dendritic cells. Moreover, our research explores the regulation of dermal TIMP3 by epigenetic factors and by cytokines known to play a role in the pathogenesis of psoriasis and thereby identifies a novel inflammatory feedback loop in psoriasis.

INFECTIOUS DISEASES

P170 | *S. epidermidis*-induced reduction of *S. aureus* skin colonization depends on the activation of distinct innate immune signaling pathways

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Our skin is constantly exposed to a large number of pathogens while at the same time undergoing selective colonization by harmless commensal microorganisms such as *S. epidermidis*. Keratinocytes, as the most abundant cell type in the epidermis, actively participate in the innate immune response by controlling its extent either by active defense mechanisms or by tolerogenic signals. The mechanism how keratinocytes discriminate commensals from pathogenic bacteria as well as how commensals influence *S. aureus* skin infection are barely understood.

This project aims at determining the influence of the skin commensal *S. epidermidis* on *S. aureus* skin infection. Furthermore, we want to analyze the activated innate immune signaling pathways in keratinocytes that modulate *S. aureus* skin infection.

Using an in vitro skin infection model with primary human keratinocytes as well as an in vivo epicutaneous mouse skin infection model we analyzed the effect of *S. epidermidis* and its secreted factors on *S. aureus* skin colonization. Additionally, we used different innate immune signaling-deficient mice to examine the signaling pathways involved in modulation of skin infection by *S. aureus*.

We show that *S. epidermidis* is able to amplify the innate immune response of keratinocytes by conditioning the epithelial surface towards pathogen defense and thereby protects the skin from *S. aureus* colonization and infection. Additionally, we demonstrate that distinct key players in innate immune signaling pathways are involved in *S. epidermidis*-mediated protection of the skin.

In healthy skin *S. epidermidis*, as part of the skin microbiota, creates a protective environment which prevents *S. aureus* from colonizing the skin. Further studies will provide deeper insight into the signaling pathways involved in commensal-induced modulation of the immune response towards *S. aureus* skin infection.

P171 | Mast cells: do they play a role in cutaneous leishmaniasis?

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The role of mast cells (MC) in hypersensitivity type I reactions and against intestinal parasites is well understood, but in parasitic skin infections—such as leishmaniasis—the role of MC is less clear. Leishmaniasis is caused by the parasite *Leishmania* (L.) *major* which is transmitted by the bite of female sandflies. In resistant C57BL/6 mice, an infection with *L. major* leads to activation of Th1/Tc1 cells with high levels of secreted IL-12p40 and IFN-gamma finally killing the intracellular parasite and long-lasting immunity against reinfection with the same *Leishmania* spp. On the other hand, susceptible BALB/c mice finally succumb to infection due to their Th2/Treg/ Th17-driven immune responses with increased levels of IL-4 and IL-10. To now analyze the role of MC in *L. major* infections, we previously showed that KitW-sh/ KitW-sh mice on a C57BL/6 background developed enlarged lesion volumes in line with increased numbers of parasites

in ears and spleens as well as higher levels of Th2-related cytokines. Whereas C.B6KitW-sh mice, bearing the kit-allele on a BALB/ c background, showed significantly decreased lesion volumes, as well as reduced numbers of local and systemic parasite burdens compared to control-mice. To now analyze mice specifically lacking only MC independent of c-kit, we assessed the phenotype of Mcpt5Cre+/-xDTA and Cpa3-Cre+/-xMcl-1fl/fl mice (both on C57BL/6 background). MC-deficient mice and control littermates were infected intradermally with physiological low doses of 1000 metacyclic *L. major* promastigotes in both ears. In contrast to KitW-sh/KitW-sh and C.B6KitW-sh mice, Mcpt5Cre+/-xDTA and Cpa3- Cre+/-xMcl-1fl/fl mice exhibited no differences in their lesion volumes compared to control littermates. Additionally, parasite burdens in infected ears and spleens showed no alterations compared to control mice and secreted levels of IFN-gamma, IL-4, IL-10 and IL-17A were comparable in all MC-deficient and control groups. Taken together, mice lacking only MC due to c-kit-independent alterations reveal that MC may in fact not contribute to disease outcome against the parasite, but that other defects associated with c-kit mutations were responsible for the prior observations.

P172 (OP02/01) | Towards vaccine development: from mice to humans—cross-reactivity of immunogenic antigens by human T cells identified in experimental leishmaniasis

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Being among the 10 most frequently occurring infectious diseases, cutaneous leishmaniasis (CL) represents a severe global problem. Inter alia, sandfly-transmitted *Leishmania* (L.) *major* parasites are responsible for the manifestation of CL. In immunocompetent hosts, healing requires the production of interferon (IFN)-γ by Th1/Tc1 cells. However, the morbidity of affected individuals with co-infections or immunosuppression is increased. Therefore, the development of an effective vaccine is of high interest. We aimed at identifying and characterizing new immunogenic pathogen-specific proteins serving as potential vaccine candidates. Thus, soluble proteins were isolated from highly immunogenic *L. major* lysate and subsequently fractionated by two-step anion exchange chromatography. Several eluted fractions induced a strong Th1/Tc1 cell-associated cytokine profile in vitro upon restimulation of primed C57BL/6 lymph node cells. Within these reactive fractions we identified 36 *L. major*-specific proteins by mass spectrometry, of which 4 proteins (90/80/17/50 kDa) were recombinantly expressed in *E. coli*. Interestingly, immunization studies in vivo with 1 μg of recombinant protein + CpG as adjuvant revealed that only the 80 kDa protein significantly promoted protection in C57BL/6 mice and susceptible BALB/c after infection with live *L. major* promastigotes. In addition, the 50 kDa protein also protected BALB/c mice from progressive disease. Protection against infection was accompanied by significantly smaller ear lesion development compared to mice treated with CpG alone, and by lower local and systemic parasite loads in C57BL/6 mice post-infection compared to infected control mice. CD4+ T cells were identified as T cell subset primarily responsible for vaccination efficacy. Depletion of CD4 cells by intraperitoneal injection of anti-CD4 antibodies during immunization of C57BL/6 mice led to a loss of protection after infection. In contrast, infected mice lacking CD8+ T cells during immunization were still protected. Finally, we determined the restimulating capacity of the 4 proteins on human peripheral blood mononuclear cells (PBMCs) ex vivo from three patients with prior *L. major* infection. As determined by incorporation of [3H]-TdR by PBMCs after incubation with recombinant protein (5-20 μg/mL) for 5d, all 4 single proteins induced proliferation of PBMCs comparable to those after incubation with total *L. major* lysate, while healthy controls PBMCs did not proliferate. Due to the coexistence of different *Leishmania* subspecies in endemic areas, the cross-protective effect against infections with different subspecies is of high interest for the vaccine development. *L. infantum* is responsible for both CL and visceral forms of leishmaniasis. Sequence alignment analysis (BLAST) revealed 87-97% amino acid sequence identity of our protein candidates to the corresponding *L. infantum* proteins. PBMCs from two *L. infantum*-infected patients showed enhanced proliferation after ex vivo restimulation with the 4 recombinant proteins compared to PBMCs from healthy donors. With regard to a favored cross-protective effect, the newly identified proteins might be potential new vaccine candidates against different forms of leishmaniasis and a source for protective T cell epitopes.

P173 | Accelerated growth of *Malassezia* species in optimized culture conditions

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Malassezia spp. is a genus of lipophilic yeasts, comprising 14 species, and the most common fungal genus on healthy human skin. It is therefore considered as commensal but also attributed a pathogenic role in skin diseases such as atopic dermatitis. The pathogenic mechanisms changing this commensal into a pathogen are not fully understood. Cultivation is a desirable tool to investigate the disease-contributing mechanisms by enabling biochemical analysis of *Malassezia* species or the investigation of interaction between *Malassezia* spp. and human skin immune cells. However, *Malassezia* spp. is fastidious, growing slowly (over 2-4 weeks) with limited yield. Growth efficacy is different between species, due to their complex and heterogeneous lipid metabolisms.

We aimed to (i) examine the lipid preferences and (ii) to optimize culture conditions of *Malassezia* species. 28 strains isolated from human skin were used for the analysis. The strains represented five species with a supposed pathogenic role in skin diseases: *M. sympodialis*,

M. restricta, *M. globosa*, *M. slooffiae* and *M. furfur*. Species identity was confirmed by sequencing. Strains were plated on various agar media (Leeming Notman, Sabouraud Dextrose, Tween 60-Esculin, Cremofur EL, mDixon) to determine their media preferences. To assess lipid preferences, commonly used culture media components (eg, tweens, olive oil, oleic acid) and human sebum components (eg, squalene, cholesterol) were added in varying concentrations.

The mono-unsaturated lipid, oleic acid was the single most effective lipid to improve growth of all investigated *Malassezia* species. Other additives provided non-essential growth enhancement. Incubation time could be shortened to 2-4 days (vs 2-4 weeks) with only slight differences between species.

In summary, we optimized media formulations for the cultivation of common *Malassezia* species with reduced cultivation time and increased efficiency. This will improve the research methods on the pathogenic role of *Malassezia* in skin diseases.

P174 | Contribution of phagocyte depletion and F4/80- and CD11b-deficiency to disease outcome in cutaneous leishmaniasis

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Cutaneous leishmaniasis is caused by protozoans of the genus *Leishmania* that parasitizes in phagocytic cells. Upon inoculation in the upper dermis by an infected sandfly, it encounters skin-resident macrophages (MΦ) and neutrophils. CR3- mediated "silent invasion" of MΦ results in parasite transformation and replication without apparent inflammatory response. Only after parasites released from ruptured MΦ infect dendritic cells (DC), clearance of infection takes place. In this phase, T cell-derived IFN-γ efficiently eliminate parasites via NO production by infected Φ. Thus, skin MΦ play a dual role in infection: early on, they provide the parasite optimal conditions for transformation and replication by shielding them from the immune system, but later on, they are also essential for subsequent killing of parasites.

First, we investigated infection outcome in F4/80-deficient C57BL/6 mice. As a specific marker for MΦ, F4/80 is expressed on most tissue resident skin MΦ. Moreover, prior studies showed an upregulation of F4/80 during MΦ maturation and a decrease in response to IFN-γ. Here, inflammatory MΦ recruited to polyacrylamide gel-induced cutaneous granulomas in F4/80-deficient mice appeared normal and were capable of *L. major* phagocytosis similar to wild-type cells. Next, to assess the exact contribution of F4/80 to disease development, we infected F4/80- deficient C57BL/6 mice. Interestingly, ko mice exhibited lesion resolution comparable to resistant C57BL/6 mice, even though the quantity of parasites per year was significantly higher at the peak of lesion. This was supported by decreased levels of IL-10, which plays an essential role in parasite persistence. IFN-γ and IL4 levels were comparable. In contrast, no alterations in frequencies of cells belonging to the innate or adaptive compartment were observed. Together, this suggests that the absence of F4/80 has no influence on disease outcome in *Leishmania* infection and the function of inflammatory MΦ.

As the infection of MΦ is predominantly mediated by CR3 (CD11b/CD18 heterodimer), we then focused on the role of CR3 in the establishment and progression of infection by using a CD11b-deficient C57BL/6 mice. Interestingly, even though the major *L. major* surface molecule lipophosphoglycan is opsonized by complement and directly binds CR3, we did not detect differences in the immune response towards an *L. major* infection due to CD11b-deficiency. Lesion development, distribution of immune cells including MΦ and release of protective cytokines was equivalent to wild-type controls.

Next, we tested whether selective depletion of MΦ alters infection outcome. To this aim, two models were used. (i) Repeated ip, administration of diphtheria toxin (DT) to mice that express the simian DT receptor under control of the LysM promotor and (ii) clodronate liposomes administered ip, and intradermally. Efficient MΦ depletion was monitored in peritoneal lavages, blood and skin. Interestingly, whenever depletion of MΦ was achieved, the survival of the mice was dramatically reduced to a few days; subsequent infections with *L. major* were impossible. If DT or clodronate liposomes were administered at fewer times (only 1×/week or only ip, respectively), then, no MΦ depletion was achieved and infection outcome was comparable to wild-type mice or control treatment as expected. Thus, due to the decreased viability of MΦ-depleted mice, an assessment of the role of Φ for *L. major* infections appeared impossible.

P175 | RNase 7 promotes TLR9 mediated sensing of self-DNA by human keratinocytes and activates an antiviral immune response

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The antimicrobial peptide (AMP) RNase 7 is one of the major AMPs secreted by keratinocytes. RNase 7 is constitutively expressed in healthy human skin and has been found to be upregulated in chronic inflammatory skin diseases such as atopic dermatitis and psoriasis. The keratinocyte derived AMPs hBD-2 and LL-37 have been described to promote TLR9 mediated activation of human pDCs by self-DNA before. Recently we observed an even stronger effect of RNase 7 in pDCs. In this study we investigated the activation of keratinocytes by RNase 7 in combination with human DNA. We detected a strong increase of IFN mRNA expression after activation of keratinocytes with RNase 7 and human DNA which was dependent on TLR9 activation. The induced IFN response activated the keratinocytes in an autocrine manner and

led to an IFN dependent upregulation of TLR3 and IFIT 1 mRNA expression, two proteins which are involved in antiviral defense. Importantly, pretreatment of keratinocytes with RNase 7 and DNA significantly reduced HSV-1 infection of human keratinocytes. The reduction of HSV-1 infection by RNase 7 and DNA was time dependent and inhibited by blocking of TLR9 and the interferon- α receptor. Furthermore, stimulation of keratinocytes with RNase 7 and human DNA induced a strong increase of IP-10 production which was dependent on TLR9 activation and downregulated by blocking of the interferon- α receptor. Production of IP-10 was further enhanced by pretreatment of keratinocytes with TNF-á. Of note, stimulation of keratinocytes with hBD-2 and LL-37 in combination with human DNA failed to induce IP-10 production. Our study demonstrates for the first time that RNase 7 functions as an alarmin by converting self-DNA released by dying host cells into a danger signal that activates human keratinocytes which maybe relevant chronic skin diseases such as atopic dermatitis and psoriasis.

PHARMACOLOGY

P176 | Penetration of polymeric nanocarriers in human skin—potential for innovations in dermatotherapy?

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Based on extensive work on nanoparticle-skin interactions we hypothesize, that polymeric nanocarriers could help improve the selectivity of topical anti-inflammatory dermatotherapy by preferred penetration in barrier-disrupted skin, targeted delivery and release at site of inflammation.

We screened a series of core-multishell (CMS) carrier loaded with dexamethasone (Dex) on ex vivo human skin using immunohistochemistry, as well as ELISA and Liquid Chromatography-Triple Quadrupole Mass Spectrometry (LC-MS) of layerwise tissue extracts. CMS carrier interact with stratum corneum (SC) of human skin in a way that Dex was delivered more effectively into deeper skin layers compared to conventional cream and that such penetration occurred slower compared to solution. High resolution X-ray spectromicroscopy also made it possible to detect the penetration pathway of Dex and nanocarriers within the lipid layers of the SC.

Similarly, we performed detailed mechanistic studies on the penetration of nanogels and were able to correlate particle stiffness to penetration properties. By comparing chemically different nanogels and combining different microscopic techniques, ie, fluorescence and electron microscopy as well as stimulated Raman spectroscopy, we could showed that nanogels can induce a perturbation of the lipids and proteins in the SC resulting in morphologic features of hydrated SC. These changes correlated well with enhanced penetration of the loaded fluorescent dye.

We found differential penetration in intact compared to disrupted skin penetration, but also show that disruption methods differentially affect such penetration despite the fact that the vast majority of nanocarrier remain in the upper stratum corneum compartments.

Altogether, these studies underline the importance of understanding the biochemical environment in those compartments which influence penetration and cargo release in more detail. A better understanding on pathways across the stratum corneum could become an important starting point for innovations in dermatotherapy.

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PHOTOBIOLOGY

P177 | Superoxide dismutase: a defense strategy of bacteria against "oxidative burst" induced by photodynamic antimicrobial chemotherapy?

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Introduction: Photodynamic antimicrobial chemotherapy (PACT) is a multi-target method to inactivate pathogen microorganisms via the excitation of a photosensitizer (PS) with visible light of appropriate wavelength in the presence of molecular oxygen ($3O_2$). There are two major pathways where reactive oxygen species (ROS) are produced. In type I (T-I)-reactions radicals like superoxide ($O_2^{\cdot-}$), hydroxyl radicals ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2) are generated via electron transfer, in type II (T-II)- reactions highly reactive singlet oxygen ($^1O_2$) is produced via direct energy transfer. Bacteria can survive under diverse oxidative conditions by appropriate bacterial stress responses in gene expression and protein activity. To understand a potential resistance/tolerance development against the "oxidative burst" induced by PACT, it is essential to study the specific defense strategies of bacteria. Bacterial key players against oxidative stress are superoxide dismutase (Sod) and catalase (Cat).

Aim of the study: In this study the efficiency rates in PACT between Gram-negative *Escherichia coli* wild type (EC WT) and its double-deleted mutant Escherichia coli ΔsodAsodB (EC ΔsodAsodB) applying two PS from different chemical classes with different 1O2 quantum yields—methylene blue and TMPyP—were investigated.

Results: The results showed that the ΔsodAsodB mutant strain is highly susceptible towards T-I-based PACT caused by Methylene blue compared to TMPyP. The lack of antioxidant enzymes is an essential factor for bacterial survival especially when T-I reaction radicals are generated, but not when exclusively singlet oxygen is produced. Furthermore, no upregulation of sodA- and sodB-genes was observed after sublethal PACT treatment, so this kind of bacterial protection defense mechanism against PACT might be harmless.

Summary: Overall the present study showed that the EC ΔsodAsodB mutant strain is more susceptible to T-I-based PACT mediated by Methylene blue, implicating that activity of Superoxide dismutase (Sod) and Catalase (Cat) might affect the antimicrobial efficacy of T-I-based photosensitizers more compared to T-II-based photosensitizers.

P178 | UVA irradiation of Senescence fibroblasts epigenetically unlock anti-apoptotic GDF15 expression via interleukin-6 mediated promoter demethylation in melanoma cells

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The incident of cutaneous melanoma is rapidly increasing in many developed countries with augmentative numbers of aged patients with a galore of evidence exist that cutaneous malignant melanoma is cogitated with exposure to sunlight; most likely to its UV component including tanning beds. Although much investigation upon aging, it's associated SASP and tumor progression has been carried in the recent years, still not much is known about UVA mediated gene alteration in elderly and its underlying mechanism contributed by senescence fibroblast. Over the past few years, strong evidence has accumulated that p53 engages also in transcriptionally regulating powerful pro-survival pathways by active transcription of genes associated with counteracting apoptosis. Among many p53 transcriptionally regulated genes, GDF15 coding for the Growth Differentiation Factor 15 (GDF15) protein most likely plays a role in regulating inflammatory and anti-apoptotic pathways in melanoma progression. However, the underlying molecular mechanisms regulating the balance between apoptosis and anti-apoptosis though p53 transcription factor is so far not fully understood. Here we report for the first time that anti-apoptotic gene GDF15 is epigenetically regulated by IL-6 upon induction with UVA. Under direct UVA exposure GDF15 is downregulated both in Senescence fibroblast and melanoma cells, but this also influences a strong UVA mediated paracrine secretion of IL-6 in senescence fibroblast, hypomethylating GDF15 gene promoter and thereby through p53-mediated transcription leads to the release the epigenetic lock in UVA exposed melanoma cells which imparts pro-survival mechanism to melanoma progression.

P179 (OP01/06) | Photodynamic therapy leads to a decrease of peripheral IL-17A+-T-cells in oral lichen planus patients

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Oral lichen planus (OLP) is a common, chronic relapsing inflammatory disorder of the mucous membranes which causes major discomfort. Currently, OLP is treated with non-specific topical or systemic glucocorticoids or immune modulators such as tacrolimus which often induce severe side-effects. In this study, we analysed the anti-inflammatory impact of photodynamic therapy (PDT) as a non-invasive, easy-to-use and safe alternative therapy option in OLP. Twenty OLP patients (mean age 62.05 ± 8.66 years) received four consecutive PDT treatments of buccal mucosal lesions within 2 weeks and peripheral T cell subsets, plasma, saliva and the size of mucosal lesions were analysed pre- and 14 days post treatment. PDT treatment led to a reduction of lesion size which strongly correlated with the decrease of CCL5 (RANTES) plasma levels (P=.004). Furthermore, upon PDT, the plasma levels of the IFN-γ-induced chemokine CXCL10 were significantly reduced (P=.003). Moreover, the number of peripheral CD4+CD137+ and CD8+CD137+ T cells were decreased (P=.06/P=.07) and strongly correlated with the expression of the chemokine receptor CCR4 (P=.002) suggesting a potential role of activated CD137+CCR4+ T cells in OLP pathogenesis. The relative percentage of peripheral T regulatory cells (CD4+CD25+CD127low), cytotoxic T cells (CD3+CD8+) and T helper cells (CD3+CD4+) was unaffected. Based on ELISPOT analysis, a significant (P=.04) decrease of peripheral IL-17A+ but not IFN-γ or IL-5+ T cells was detected. Noteworthy, IL-17A+ T cells were found adjacent to the dermal-epidermal junction in LP mucosal lesions. Overall, the inflammatory T cell infiltrate in OLP lesions was not significantly reduced by PDT treatment within 14 days but there was a decrease of lesional CD4+ T cells. These results show that PDT treatment of OLP lesions exerts systemic anti-inflammatory effects leading to a reduction of pro-inflammatory CD137+ and IL-17A+ T cells and T cell recruiting chemokines such as CXCL10.

P180 | Mechanism of action of phenalen-1-one bactericides compared to benzalkonium chloride

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Antimicrobial agents like n-alkyl-dimethylbenzylammonium chloride (BAC) exhibit problems like insufficient efficacy against bacteria combined with adverse effects on eukaryotic cells. However resistances against such antiseptics are increasing. Therefore, alternative antimicrobial approaches like the photodynamic inactivation of bacteria (PIB) may be favorable. PIB means that irradiation with visible light (no UV) of the so-called photosensitizer leads to production of reactive oxygen species that kill bacteria via an oxidative burst.

Recently our group introduced phenalen-1-one derivatives as a novel class of photosensitizers (PS) based on plant phytoalexins. These PS demonstrated promising properties for PIB application in both dental and dermatological practice.

In view of structural similarity to BAC (n-alkyl-dimethylbenzylammonium chloride with C8 to C18 atoms) new phenalen-1-one derivatives were synthesized. A similar chemical motif compared to BAC can be generated by introducing variable C8 or C12 alkyl substituents in the PS structure to narrow down the know BAC effect and the antibacterial PIB effect depending on the chemical structure.

The aim of this in vitro study was to evaluate the antibacterial efficacy of five synthesized perinaphthenones (PN) with elongated alkyl chains in comparison to BAC as well as the steric influence of the chemical head groups for penetration in lipid membranes. Furthermore, we investigated a possible effective concentration range for five PN compounds and BAC, at which antimicrobial efficacy (≥5 log10 reduction of CFU) coincides with eukaryotic cell survival (≥80% NHEK survival). All new PN derivatives exhibit pronounced antimicrobial efficacy at concentrations where keratinocytes are only marginally affected. Effective concentration ranges were found for four out of the five photoactive PN derivatives, but not for BAC and the compound with the BAC-like alkyl-chain. Furthermore, we could show for the first time that besides the alkyl-chain length the size and polar area of the respective head groups of phenalen-1-one derivatives or BAC had a great influence on the incorporation inside lipid membranes, thereby affecting its antibacterial efficacy.

P181 | Effect of UV-R on skin microbiome and its interference in UV-induced immune suppression

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Ultraviolet-radiation (UV-R) is long known to have an intense effect on skin and its components, leading to activation of the innate immune system and ultimately suppressing the adaptive immune response in healthy individuals. This modulation of the immune system can be both beneficial and harmful. The skin hosts innumerable number of bacteria, fungi, viruses, archaea and mites, and to date very little is known about the effects of UV-R on the skin microbiome. In this study we looked at the effect of different single doses of UV-B on the skin microbiome of the mouse at different time points and performed qPCR to determine expression levels of various antimicrobial peptides (AMPs). We further employed the model of induction of contact hypersensitivity (CHS) on disinfected (with local antiseptics) and germ-free mice to study interference of the microbiome in UV-induced immune suppression. We observed increased number of species at lower dose and reduced number at higher dose compared to control (UV-unexposed) mice. Interestingly, beta diversity analysis showed significant variation in microbial abundance at different doses and time points. We further performed LDA effect size (LefSe) analysis and observed abundance at taxonomic levels. qPCR for various AMPs showed differential expression levels suggesting that UV-B profoundly modulated the skin microbial load either directly or via expression of AMPs and affected its recolonization potential. Intriguingly, germ-free mice showed much higher levels of suppression of CHS (71%) compared to SPF mice (41%). A similar trend of more suppression of CHS was observed in disinfected mice compared to control mice. Collectively, our results suggest that the skin microbiome is sensitive towards UV-R and profoundly influences UV-induced immune suppression.

P182 | UVA radiation changes gene expression and processing of Laminin-332 in human skin cells

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UVA radiation with their longer wavelength is a component of visible sunlight, however, it substantially increases the risk of skin damage. UVA is associated with wrinkle formation, reduced recoil capacity, blister formation, increased fragility, and impaired wound healing all of which can be seen in photoaging. Exposure to UVA radiation results in marked changes in the connective tissue of the dermis and epidermis, which are due to quantitative and qualitative alterations of the surrounding extracellular matrix (ECM).

Laminins are major components of the basement membrane, which is a thin ECM layer that separates the epithelium from underlying connective tissue. The skin and its surrounding cells respond to UVA damage by causing the formation of free radicals (ROS), solar elastosis, and an increase of matrix metalloproteinase (MMP) activity. Intense solar stimulation leads to a degradation of ECM and an abnormal ECM organization.

In this context, we wanted to analyze the role of Laminin-332, one of the major ECM components in human skin keratinocytes. We studied the effect of UVA radiation on Laminin-332 expression and secretion focusing on laminin α3 and its C-terminal tandem module LG4-5. We show that UVA radiation leads to a slight upregulation of LM-332 in keratinocytes, whereas it downregulates the secreted α3 chain and its secreted LG4-5 tandem module. In contrast, we observed downregulation of intracellular Laminin-511. As control fibronectin was used, which showed decreased expression upon UVA radiation.

For the first time, our study provides new insights into the different behaviour of Laminin-332 in human keratinocytes due to UVA radiation. While secreted laminin α3 and LG4-5 decrease after UVA

exposure, we observe an increase of cellular LM-332 that may serve as a protective mechanism.

P183 | Ultraviolet (UV)-A irradiation induces melanoma invasion via enhanced Warburg effect

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Melanoma is a malignant tumor with high mortality and increasing prevalence for which exposure to ultraviolet (UV) radiation is considered to be an important risk factor. Especially UVA (320-400 nm) radiation induces the formation of reactive oxygen species (ROS) which oxidatively damage cellular molecules. It was recently shown that UVA radiation is capable to induce murine melanoma, but the role of UVA in the progression of melanoma is still not investigated. During early progression of melanomas before metastasizing, most melanomas show initial proliferation of melanoma cells and a metabolic characteristic of most proliferating tumor cells is the preference of aerobic glycolysis instead of oxidative phosphorylation (Warburg effect).

Here we investigated the role of UVA radiation in progression of melanoma, especially induction of progression markers, changes in Warburg effect, invasive potential and metabolism.

Upon UVA radiation, initial melanoma cells show increased Warburg effect with increased glucose consumption and increased lactate production. With in vitro invasion assays we show, that lactate, which is produced via UVA enhanced Warburg effect, increases invasiveness of initial melanoma cells. This effect is mediated by reactive oxygen species which are induced by UVA radiation, as treatment with ROS scavengers impairs UVA induced lactate production and invasion. Furthermore transcription of tumor relevant matrix metalloproteinases and not TIMP1 is highly upregulated upon treatment with lactate. Therefore we could show in melanoma cells, derived from melanomas of early progression that production of lactate, induced by UVA radiation, increases invasiveness of initial melanoma cells via expression of MMPs. Furthermore we found that UVA radiation also changes the consumption of other metabolites like tyrosine and phenylalanine. This shows that not only metabolites of the Warburg effect, but also other metabolites are changed upon UVA radiation.

P184 | Interplay between the innate immune system and environmentally induced aging

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The skin is the first line of defense not only against pathogens but can also react to sterile danger signals such as UVB-irradiation. Acute exposure to UVB irradiation leads to skin inflammation, whereas chronic exposure leads to skin aging and tumor formation. Toll-like receptor (TLR) activation via MyD88 is crucial for recognition of pathogens but also plays a role in UVB-induced inflammation. We aim to analyze whether the activation of the innate immune system via MyD88 contributes to UVB-induced skin immunity and to extrinsic skin aging. We induced extrinsic skin aging using a 6 week chronic UVB-irradiation model in vivo comparing wild-type, MyD88-deficient mice and mouse strains selectively expressing MyD88 either in keratinocytes, CCL17-positive dendritic cells (DC) or macrophages and neutrophils.

Chronic UVB-irradiation leads to epidermal hyperplasia and an increase of dermal mast cell numbers, but did not lead to the induction of inflammation. MyD88-deficiency did not contribute to the UVB induced tanning response, but lead to enhanced DNA damage such as formation of cyclobutane pyrimidine dimers (CPDs). MyD88-signaling in myeloid cell populations attenuated UVB-induced CPD levels to levels in WT mice, while the exclusive expression of MyD88 in keratinocytes further enhanced CPD formation. MyD88-deficient mice showed reduced epidermal thickening and attenuated dermal mast cell numbers compared to wild-type mice. While expression of MyD88 exclusively in macrophages and neutrophils did not contribute to epidermal thickening, expression of MyD88 in keratinocytes or CCL17- positive DC contributed to epidermal thickening.

These data indicate that MyD88 influences hallmarks of UVB-induced extrinsic skin aging such as epidermal hyperplasia and dermal mast cell numbers, which seemed to be independent on induction of inflammation. Expression of MyD88 in distinct skin cell populations contributes differentially to these effects. Furthermore we could show that MyD88 is involved in the DNA damage response, which could possibly link innate immune signaling and UVB-induced cell damage and may influence UVB-induced tumor formation in a MyD88-dependent manner.

PRURITUS

P185 | Role of dysesthetic sensations in chronic pruritus

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Chronic pruritus (CP) is a major symptom in various diseases. It is often combined with additional dysesthetic sub-sensations such as cold, warm, stinging or burning. So far, little is known about the origin or the causes of such sub-sensations. Perception of thermal sensations may be

associated with the expression of thermosensitive channels. Here, members of the TRP family are most likely involved as they have been recently found to be associated with itch perception and CP. On the other hand, architecture, sensitization and function of nerve fibers may be involved in CP with these dysesthetic perceptions, too. Within this study, we therefore aimed to investigate the expression of different thermosensitive channels, the intra epidermal nerve fiber density (IENFD) and the reaction on itch evoking stimuli in respect to such sub-sensations.

Thirty-one patients suffering from CP and 18 healthy controls (HC) were included in this study. Of the patients, 18 showed the sub-quality warmth (CP-W) and the remaining 13 reported stinging/burning (CP-SB). Immunohistochemistry and quantitative PCR were used to investigate expression of thermosensitive channels. IENFD was measured by microscopic evaluation of PGP9.5 staining on skin sections. Perception of mechanical and thermal stimuli was quantified by quantitative sensory testing (QST). Erythema and redness was measured after application of capsaicin (lotion) or histamine (intracutaneous injection).

Both, capsaicin as well as histamine application evoked different reactions in HC and CP. Whereas HC showed more itch sensation after capsaicin application CP were characterized by a stronger burning sensation. Histamine provoked more wheal and erythema in HC but CP patients reported more itch. By means of QST higher warm detection thresholds were detected for CP(-W) and higher mechanical pain sensitivity in CP(-SB). The first one indicates disturbed C-fiber function and a peripheral effect whereas the latter one points towards Aδ-fiber sensitization and a generalized effect. PGP9.5 staining revealed reduced IENFD in CP(-W) making neuropathic events in CP very likely. We found higher expression of TRP channels in CP with different pattern for CP-W (upregulation of TRPV1, TRPV2, TRPM8) and CP-SB (upregulation of TRPV4).

Peripheral sensitization and disturbed function of C- and Aδ-fibers are relevant in CP with clinical presence of dysesthetic sensations. Combined peripheral and central effects are present in these patients and may provoke different sub-sensations. Furthermore, neuropathic mechanisms play an important role in this type of CP. Higher expression of TRP channels in CP and different pattern in CP subgroups indicate their relevance for both, CP and different sub-sensations.

Taken together, subgroups of CP patients defined by different dysesthetic subsensations may differ in the involvement of C and Aδ-fibers, the central and peripheral sensitization and the expression of TRP channels, too. The investigation of the implications for therapeutic approaches in these subgroups is urgently needed.

P186 | Body heatmaps of pruritus in dermatological diseases

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Many dermatological disorders are associated with pruritus. While in some diseases like urticaria, atopic dermatitis or lichen planus, itch is a hallmark symptom, in other diseases pruritus is described to be present sometimes or often. In recent years, there were various reports about the prevalence of itch, both in the general population and in some selected skin diseases. A detailed characterization of the presence, intensity and localization of itch in different skin diseases and in patients with pruritus without affected skin is, as of yet, missing.

Here, we have analyzed characteristics of chronic pruritus, with a special focus on itch distribution patterns, revealing body heatmaps of pruritus in different skin conditions.

Unselected, consecutive patients with active dermatologic disorders, that can be, reportedly, associated with pruritus and control patients with non-itchy angioedema were invited to participate. 880 in- and out-patients of the department of dermatology at Charité – Universitätsmedizin Berlin, with 19 different dermatological diagnoses have given informed consent and filled out study questionnaires. Patients with chronic spontaneous urticaria (n=143), psoriasis (n=138), atopic dermatitis (AD) (n=129), chronic inducible urticaria (CINDU) (n=76), prurigo (n=75), cutaneous T cell lymphoma (CTCL) (n=68), angioedema (n=64), mastocytosis (n=54), pruritus on unaffected skin (n=30), parapsoriasis en plaque (n=29), cutaneous B cell lymphoma (CBCL) (n=26), bullous pemphigoid (BP) (n=15), lichen planus (n=11), and 6 other diseases with <10 patients were included. All patients were asked to mark on two silhouettes of a human body (front and back) the area on which any pruritus usually occurs and on two other silhouettes the area on which the maximum pruritus usually occurs. These data were transferred to a digital form and they were further processed digitally to create color-coded heatmaps of pruritus distribution. Additionally, other pruritus characteristics such as average and maximum itch intensity were recorded.

Previous chronic pruritus and current pruritus (within the last week) were reported by 100% and 76% patients with CSU, 88% and 74% psoriasis, 100% and 91% AD, 100% and 79% CINDU, 100% and 96% prurigo, 48% and 28% CTCL, 81% and 56% mastocytosis, 100% and 83% pruritus on unaffected skin, 48% and 28% parapsoriasis en plaque, 38% and 23% CBCL, 100% and 67% BP, 82% and 73% lichen planus patients. Among diagnoses with at least 10 patients included, the most intense maximal pruritus was reported by patients with pruritus on unaffected skin (mean SD of maximum visual analogue scale [VAS] =8 ± 1.4), followed by AD (7.5 ± 2.2), prurigo (7 ± 2.4), CINDU (6.7 ± 2.2), BP (6.5 ± 3.0), CSU (6.4 ± 2.7), psoriasis (6.1 ± 2.7), lichen planus (5.7 ± 3.8), CTCL (5.6 ± 2.9), parapsoriasis en plaque (5.4 ± 2.3), mastocytosis (5.1 ± 2.2) and CBCL (4.1 ± 2.5).

Taken together, we have visualized, for the first time, the localization of average and maximum pruritus in patients from a large variety of dermatological diseases. Together with itch intensity, the results show a characteristic pattern of pruritus for many diseases. This can lead to a better understanding of the pathophysiology of itch in these diseases, help in the development of better treatment options and can lead to a better management of our patients.

TUMOR BIOLOGY

P187 | Stat1-dependent cancer cell senescence controls cancer development during Th1 driven immunotherapy

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Immunotherapy with tumor-associated antigen (TAA)-specific T-helper-1 (Th1) cells mediates anti-tumoral effects in patients with skin cancers, such as melanoma or squamous cell carcinoma. Similarly, adoptive transfer of TAA-specific Th1 cells prolongs the life of transgenic tumor-bearing mice by induction tumor cell senescence. Cancer immunotherapies with monoclonal antibodies against exhaustion-associated surface molecules, known as immune checkpoint inhibitors, reactivate T cells and have durable therapeutic efficacy in patients with skin cancers and various other types of cancer. Therapies with immune checkpoint inhibitors improve the prognosis of patients, immune checkpoint inhibitors first kill cancer cell and then induce a state of dormancy in the remaining metastasis. While the killing mechanisms causing cancer regression have been described, the mechanisms causing the long-lasting tumor dormancy remain unknown. To investigate the mechanisms of tumor dormancy established by immune checkpoint inhibitors we analyzed the therapeutic monoclonal antibodies in RIP-Tag2 mice 4 weeks prior to cancer-induced death. Expression of the large T antigen (Tag) under the rat insulin promotor (RIP) leads to pancreatic islet cancers. The therapy of this advanced islet cancers with the immune checkpoint inhibitors PD-L1/LAG-3 (Programmed-Death- Ligand-1/Lymphocyte-Activation Gene 3) and tumor-specific Th1 cells restored normal blood glucose, caused a p16INK4a-positive and Ki67-negative senescent phenotype in the remaining cancer cells, and restored long-term survival of the mice. This was strictly dependent on an intact IFN/Stat1-signaling pathway in the cancer cells. Cancers of RIP-Tag2xStat1.ko mice, deficient in interferon-γ-signaling, did not respond, neither to the therapy with Tag-specific Th1-cells nor to the combined therapy with Th1-cells and immune checkpoint inhibitors. Importantly, T-cells were found in all treatment groups, but p16INK4a induction and Ki67 suppression was absent from the Stat1-deficient cancers. Furthermore, RIP-Tag2xStat1.ko cancer cells were electively resistant to interferon-induced senescence but fully susceptible to apoptosis or T cell-mediated killing, both in vitro and in vivo.

Taken together, our data demonstrate that combined Th1-cell and immune checkpoint inhibitor therapy are capable to arrest tumor growth of advanced cancers. Yet these cancers require a functional Stat1-signaling pathway to induce senescence.

P188 | PD-L1 expression and composition of tumor microenvironment in primary cutaneous diffuse large B-cell lymphoma

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In systemic diffuse large B-cell lymphoma (DLBCL) PD-L1 expression has been found in up to 24% of the tumors, depending on the examined material, the antibody and the subtype of DLBCL (GCB vs ABC). PD-L1 expression in primary cutaneous DLBCL has not been investigated so far. We investigated 16 paraffin-embedded tissue samples of pcDLBCL (13 leg-type (LT), 3 other-type (OT)) for their PD-L1 expression by immunohistochemistry (Clone SP142). Moreover, the tumor microenvironment was studied, using the antibodies against PD-1, PD-L1, CD33, CD68 and CD163.

We observed a membranous expression of PD-L1 within the tumor cells in all of our investigated cases (all: mean 19.9%; LT: mean 19.16%, OT mean: 23.3%). Among DLBCL-LT tumors, 10 cases were sub-classified as ABC-type and 2 as GCB type, with a lower PD-L1 score in GCB-type. The surrounding infiltrate was brisk, consisting predominately of M2-macrophages and CD33+ cells, including myeloid derived suppressor cells (MDSC). As described before, T-cells were only a minor component. The number of PD-1 expressing tumor infiltrating lymphocytes (TIL) differs not significantly between OT and LT. Myeloid derived cells presented in 63% a PD-L1 co-expression. CD68+ macrophages present only a minor component of the tumor, but we found a very high number of CD163+ tumor associated M2-macrophages (TAMs) admixed. The number of MDSC and the number TAMs were not correlated with the different subtypes of DLBCL.

We postulate that PD-L1+ tumor cells and MDSCs shield the tumor against attacking TILs, by induction of apoptosis. This might explain the low number of PD-1+ TILs in DLBCL and could be an additional explanation of the poor prognosis in this disease. An anti-PD-1 (and anti-PD-L1) antibody therapy seem to be a promising therapeutic approach for this aggressive form of cutaneous B-cell lymphoma.

P189 | The role of PD-L1 in tumor microenvironment of merkel cell carcinoma

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Merkel cell carcinoma (MCC) expresses PD-L1 in variable degree, depending on detection of merkel cell polyomavirus (MCPyV). Some studies have already investigated tumor microenvironment (TME) in MCC, focusing on tumor-infiltrating lymphocytes (TILs).

We investigated 14 paraffin-embedded tissue samples of MCC and stratified them by their detection of MCPyV (8 MCPyV(+) and 6 MCPyV(−) cases). Next to PD-L1 and PD-1 expression in the tumor-cells, the microenvironment was characterized by immunohistochemistry for PD-1, PD-L1, CD33, FOXP3 and MXA.

We observed a membranous tumoral PD-L1 expression in 7/8 MCPyV(+) samples, with a low median number of positive tumor cells (mean 2.7%). The PD-L1 staining showed an "interface" distribution. 2/12 tumors were also positive for PD-1 (almost all tumor cells). The tumor cells were surrounded by a shield of CD33/PD-L1-expressing cells, which included myeloid derived suppressor cells (MDSC). Expression of PD-L1 by tumor cells was higher in areas with a denser immune infiltrate and a higher PDL1 expression in TME. Moreover, a high level of an interferon-inducible protein (MXA) was detectable in these areas, too.

CD33(+) cells without direct tumor contact were PD-L1 negative. Only a low number of FOXP3(+) regulatory T-cells were admixed (12% of TME), in only 6% PD-L1 is simultaneously expressed. Tumor cells of MCPyV(−) samples were predominately PD-L1 negative.

We assume that PD-L1/CD33(+) cells (including MDSC) shield the tumor against the tumor defending PD-1(+) immune cells. Our data imply that a tumor-immune interaction is necessary to induce PD-L1 expression in the tumor cells and the TME. Interferon seems to play a role in this interaction.

P190 | Altered balance of p53 family member interplay in malignant melanoma

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Majority of melanoma cells exhibit wild-type p53 with impaired functional activity. The restoration of its tumor suppressor function can benefit the melanoma therapy. Interestingly, different C- and N-terminal isoforms of the p53 family member p73 can confer either tumor-suppressive or apoptosis suppressive function. In melanoma cells, the functional role of C-terminal p73 isoforms is widely unknown.

We could show that endogenous expression of different p73 isoforms are altered during the developmental stages of melanoma and during the resistance acquisition towards MAPKi treatment and observed a predominant expression of C-terminal p73α isoform. Furthermore, we found a distinct crosstalk of different p53 family member activity in response to melanoma cell treatments. Interestingly, we could show that the down-regulation of endogenous p73 expression was sufficient to induce apoptosis and viability decline of metastatic melanoma cell lines. The endogenous level of p73 isoforms was also relevant for the sensitivity towards DNA damaging treatments such as chemotherapy or ionizing radiation especially in MAPKi resistant metastatic melanoma cells.

Our data let assume that the interplay between different p53 family member isoforms is critical for p53 dependent melanoma treatment response. We also propose that specific C-terminal p73 isoforms are involved in the mediation of therapy resistance.

P191 (OP01/05) | Emerging targets in resistance to MAPK inhibition: tackling the RSK in malignant melanoma

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The clinical availability of small molecule inhibitors specifically targeting BRAF mutated at V600 marked a significant breakthrough in the therapy of melanoma carrying such mutations. Despite a dramatic anti-tumour activity and improved patient survival, rapidly emerging resistance to these inhibitors, however, greatly limits their clinical benefit. A large number of different resistance mechanisms have already been described, yet common to many of them is the reactivation of the MAPK signalling pathway. The p90 ribosomal S6 kinase (RSK) is a downstream effector of the MAPK signalling cascade and has been reported to enhance survival of melanoma cells in response to chemotherapy. Based on that, the aim of this study was to asses a potential role of the RSK in resistance to the BRAFV600E/K inhibitor vemurafenib.

Comparing melanoma cell lines and patient tissue with acquired resistance to vemurafenib to their sensitive counterparts reveals a significantly enhanced activity of the RSK in the resistant cells, which seems to be mainly based on elevated MAPK signalling. In fact, RSK inhibition markedly impairs the viability of vemurafenib resistant melanoma cells and seems to be effective both in 2-dimensional and in 3-dimensional culture systems, especially when applied over a longer time period. The effect of RSK inhibition can be partly reproduced by downregulation of the Y-box binding protein 1 (YB-1), an important target of the RSK. Intriguingly, RSK inhibition also retains its efficacy in melanoma cells with combined resistance to vemurafenib and trametinib.

These data suggest that active RSK signalling might be an attractive, novel therapeutic target in melanoma cells with acquired resistance to MAPK pathway inhibitors.

P192 | Variant MCC cell lines are more closely related to SCC than to classical MCC cell lines

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Merkel cell carcinoma (MCC) is an aggressive skin cancer—recent results suggest that it is also a very heterogenous skin cancer. While in at least 80% of all MCCs the Merkel cell polyomavirus (MCPyV) is present and presumably involved in its carcinogenesis, in a subgroup of MCPyV negative MCCs based on DNA mutation signatures a UV-carcinogenesis is assumed. This dichotomy also translates into the characteristics of MCC cell lines. While classical MCC cell lines show a neuroendocrine, ie, as spheroids, growth patterns, variant MCC cell lines show an adherent growth pattern. To understand these differences, we compared the variant MCC cell lines MCC13 and MCC26 to six classical MCC and four squamous cell carcinoma (SCC) cell lines in terms of morphological features, protein, mRNA and microRNA expression. Morphology of cell lines was captured by immunofluorescent stainings of MCC and SCC specific markers. mRNA expression profiling was done by nCounter PanCancer Pathway panel (detecting 770 genes) and microRNA expression profiling was performed using the nCounter human v2 miRNA panel (detecting 800 mircroRNAs).

Morphologically, MCC13 and MCC26 obviously shared more similarities with SCC than with classical MCC cell lines. Moreover, mRNA and microRNA expression patterns of variant MCC cell lines clustered with SCC and not with classical MCC cell lines. While classical MCC cell lines highly expressed genes involved in chromatin modification and DNA repair, variant MCC and SCC cell lines overexpressed genes involved in oncogenic Ras and PI3K pathways.

In summary, our data suggest that variant MCC cell lines are closer related to SCC than classical MCC cell lines.

P193 (OP03/01) | Prognostic significance of PDCD1 (PD-1), CD274 (PD-L1) and PDCD1LG2 (PDL2) promoter methylation for melanoma patients

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Background: The programmed death-1 (PD-1) receptor (PDCD1) and its ligands programmed death-ligands 1 (PD-L1/CD274) and 2 (PD-L2/PDCD1LG2) play critical roles in T cell exhaustion and tolerance. It has been shown that overexpression of PD-L1 and PD-1 on tumor cells and tumor infiltrating lymphocytes correlates with poor prognosis in melanoma. The tumor intrinsic upregulation of immune checkpoint receptors and ligands is not fully understood. We hypothesize that the expression of PD1 and its ligands are subject to regulation via promoter methylation and that promoter methylation of genes constituting the PD-1 axis may be of predictive value for PD-1 checkpoint inhibition.

Methods: We analyzed 449 melanoma tissues from the "The Cancer Genome Atlas (TCGA)" for gene methylation and gene expression of PDCD1, CD274 and PDCD1LG2 with regard to melanoma-specific survival. To validate these data we determined DNA methylation in 98 formalin-fixed paraffin-embedded cutaneous melanomas by a quantitative methylation-specific qPCR assay targeting the promotor regions of PDCD1, CD274 and PDCD1LG2. We used the published gene methylation data set of 16 patients treated with anti-PD1 antibodies (Hugo et al. 2016) to analyze the predictive value of promoter methylation of genes constituting the PD-1 axis for anti- PD1 response.

Results: In the univariate Cox proportional hazard model, high CD274 and PDCD1LG2 methylation were associated with adverse outcome in the TCGA cohort. For PDCD1 methylation, no significant prognostic power was found. However, in cutaneous metastases PDCD1 and CD274 methylation served as a prognostic factor. Accordingly, patients with highly CD274 methylated melanoma metastases showed a significantly worse melanoma-specific survival. The prognostic value of dichotomized CD274 methylation was further confirmed by Kaplan-Meier analyses. For PDCD1, an adverse outcome was observed in patients with hypermethylated metastases. For PDCD1LG2, no such association was observed. In the validation cohort, dichotomized PDCD1 methylation was significantly associated with shorter progression-free survival. For CD274 methylation, a trend towards shorter progression-free was observed. No such association was observed for PDCD1LG2. A χ^2 analysis of 16 patients treated with anti-PD-1 antibodies, showed that a complete response was significantly associated with methylation of CD274 and showed a trend for PDCD1LG2 methylation.

Summary/Outlook: Our results imply that PD-1, PD-L1 and PD-L2 mRNA may be subject to epigenetic promoter control in melanoma. CD274 promoter methylation, potentially in combination with PDCD1LG2 methylation, might serve as a predictive biomarker for the response to immunotherapies targeting the PD-1/PD-L1 axis. Their analysis should be considered as a companion biomarker, and we would therefore recommend the integration of its analysis in running clinical trials.

P194 | Non-invasive in-vivo visualization of melanoma micrometastases in sentinel lymph nodes

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The lymphoscintigraphy with 99 m-Technetium is the present gold standard to detect metastases in the first draining lymph node (SLN) of patients with melanoma. It is a radioactive and cost-intensive surgical intervention with potential morbidity which does not show a statistically significant advantage in terms of overall survival. Novel imaging techniques to prevent surgical intervention could replace conventional lymph node excision protocols. The multispectral optoacoustic tomography (MSOT) offers a promising approach to non-invasively assess lymph node metastases in the SLN.

MSOT is a hybrid imaging technique, combining high-resolution ultrasound with molecular specific optical excitation. Pulsed light of time-shared multiple wavelengths illuminates the tissue of interest and

establishes transient photon fields in tissue. In response to the fast absorption transients by tissue elements, acoustic responses are generated via the photoacoustic phenomenon, which are then detected with acoustic detectors. Light of different wavelengths is selected to target the absorption transient of the chromophore or fluorochrome, as selected for spectral differentiation.

In our first in-human study*, we present the accuracy and high sensitivity of detecting metastases in SLN of patients with melanoma by non-invasive multispectral optoacoustic tomography (MSOT). Unfortunately, in our trial, the detection of melanin as indirect indicator for melanoma metastases in lymph nodes suggests a high false positive rate of lymph node metastases. The specificity to determine lymph node metastases in vivo by means of MSOT shall be enhanced by detecting a fluorescent antibody attached to Melanoma-associated chondroitin sulfate proteoglycan (MCSP) on melanoma cells. MCSP is a cell-surface antigen expressed on more than 85% of all melanoma cells. As experimental setting, we use resected SLN of patients with melanoma directly after extirpation. Former studies showed the continuous lymphatic perfusion of the lymph node for several minutes after resection. We applicate an "untargeted" fluorescent tracer and the "targeted" fluorescent anti-MCSP antibody into the afferent lymphatic vessel. They are injected simultaneously and in equal concentration. After enrichment in the SLN, the anti-MCSP antibody specifically attaches to melanoma cells. Due to their equal biodynamics, the untargeted tracer equally enriches in departments where the MCSP accumulates non-specifically. After correlation of the two fluorescent signals, the signal of the antibody against MCSP is solely presented in MSOT and indicates the presence of melanoma cells.

As the antibody against MCSP is not yet approved for human trials, we have established a mouse model to detect human melanoma cells in lymph nodes in-vivo. To enhance the transferability of our model, we subcutaneously transplanted the human melanoma cell lines M24met and MV3 in NOD SCID mice. Approximately 4-6 weeks after transplantation lymph node metastasis occurred. According to the above-mentioned protocol, we injected both an untargeted tracer and the targeted anti-MCSP antibody peritumorally. The sensitivity and specificity of in-vivo determination of lymph node metastases by MSOT is currently under investigation.

The establishment of a pre-clinical in-vivo MSOT model for non-invasively detecting melanoma cells in the SLN of NOD SCID mice will help to translate a non-invasive in vivo visualization of micrometastases in melanoma patients.

*Stoffels et al., Sci Transl Med 2015, Metastatic status of sentinel lymph nodes in melanoma determined noninvasively with multispectral optoacoustic imaging.

P195 | Deciphering tumour heterogeneity towards optimised therapy selection in malignant melanoma

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Introduction: Targeted therapies have become a mainstay of melanoma therapies. However, resistance or heterogeneous responses to these therapies is diminishing their clinical success. One explanation for this phenomenon is that tumours contain diverse clones driven by different driver mechanisms causing varying responses to therapy. This tumour heterogeneity is an unsolved problem for the selection of appropriate therapies. In this first phase of our project we aimed to estimate whether and how intra-tumour heterogeneity can be mapped by in depth whole proteome analysis.

Methods: Two metastases from two patients were split in four and five parts respectively. Each of these parts was subsequently analysed using MS/MS-based label-free shotgun proteomics with one technical replicate. Spectra were identified using the search engine MSGF+ and clustered using our novel spectra-cluster algorithm (J. Griss et al., Nat. Methods 2016). The clustering results were used to improve the accuracy of the label-free quantitation.

Results: On average, 2500 proteins were identified per metastasis piece. Using the clustering results significantly reduced the observed technical variability. As expected, the predominant factor of variability was the two different patients with 698 proteins differentially expressed between them. A pathway analysis revealed that while one patient had a predominantly innate immune response, the other patient showed a strong adaptive immune response (ie, changes in MHC 1 and 2 complex, IFN and NFkB-related proteins). Additionally, one patient showed a strong upregulation of proteins responsible for extracellular matrix degradation and adherens junctions.

Conclusions: These experiments show that we are able to identify central processes to cancer biology using proteomics technologies. Additionally, through our in-depth analysis we are able to pinpoint regional changes of these altered pathways within a single metastasis and thereby assess the influence of intra-tumour heterogeneity on future projects.

P196 | Single-cell RNA-seq analysis of patient-derived melanoma cultures

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Cellular heterogeneity is regarded as a major reason for the variable tumor responses to targeted treatment and early recurrences under treatment. Single-cell genomics technologies allow unprecedented insights into the cellular heterogeneity of tumor samples which opens the possibility of finding new targets for treatment. Recently, we presented data from a single-cell RNA-seq study of a BRAF/NRAS wild-type melanoma metastasis. By using self-organizing maps we

identified different sub-populations defined by gene expression modules involved in cellular proliferation, oxidative phosphorylation, pigmentation and cellular stroma. Genomic heterogeneity of melanoma samples was further substantiated by the analysis of 2 melanoma samples of BRAF- and NRAS-mutant melanomas, which showed partly overlapping gene expression modules, but also additional modules of genes involved in the interferon/inflammatory response and epithelial-mesenchymal transition. Interestingly, some of the gene expression modules had prognostic relevance when compared with gene expression and patient survival data from a series of independent gene expression studies of tumor biopsies, which supported the finding that single-cell analyses may detect clonal or subclonal structures in melanoma cultures which are also active in melanoma tumors. Since CDK4 was consistently highly expressed in the majority of cells of the BRAF/NRAS wild-type melanoma, melanoma cells of this tumor were treated with CDK4 inhibitor palbociclib, which resulted in a significant treatment response. Finally, we identified a low abundant sub-population that highly expressed a module containing ABC transporters and multiple aldehyde dehydrogenases (ALDHs), which are regarded as markers for melanoma stem or initiating cells. Taken together, our results describe heterogeneity in melanoma short-term cultures which might be relevant for patient survival and treatment response.

P197 | Immunogenic cell death of B16 melanoma cells following exposure to cold physical plasma derived oxidants

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Although advances in cancer immunotherapy have shown promising clinical results, metastatic melanoma is still associated with poor prognosis in some patients. Especially non responders show a low-grade inflammatory profile in the tumor microenvironment. Reactive oxygen and nitrogen species are known to facilitate redox signaling and modulate inflammation and tumor immunosenescence. A new technology to generate these species in a highly controlled and localized manner is cold physical plasma. These ionized gases expel reactive molecules while not delivering thermal damage to the tissue. In a murine melanoma model it has been recently shown that melanoma growth was decelerated via treatment with cold plasma. Yet, molecular investigations deciphering molecular consequences in plasma treated melanoma cells are scarce. B16F10 murine melanomas were treated with cold plasma, and metabolic activity, apoptosis induction, and release of damage-associated molecular patterns (DAMPs) was monitored. Plasma oxidized tumor cells which was paralleled by intra and extracellular expression of peroxiredoxins. Overall metabolic activity was reduced by induction of immunogenic apoptosis as presence of CRT and HMGB1 suggested. Finally, co-culture of plasma-treated melanomas with splenocytes showed increased calcium signaling in the latter as well as a modulated cytokine signature. As such, cold physical plasma may be a palliative alternative in advanced stage melanomas and possibly an adjuvant tool in cancer immunotherapy.

P198 | Exogenous oxidants and macrophage polarization in melanoma cells

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Macrophages are important regulators of inflammation which is crucial in many pathologies such as cancer. Specifically, inflammation is fine tuned by the polarization state of macrophages (eg, M1, M2a, M2b, and M2c) on the one hand as well as the oxidant and antioxidant environment on the other. As such, exogenously derived oxidant may be able to alter the macrophage state which may be of relevance in the tumor setting. Therefore, macrophage polarization was investigated in the tumor context in vitro. M0 or polarized THP 1 or human peripheral blood monocytes/macrophages were co-cultured with SK Mel-28 melanoma cells either in a direct approach or using a transwell system. Changes in polarization were assessed by fluorescence microscopy and flow cytometry in unchallenged and oxidatively damaged melanoma cells. Oxidants were generated using a cold physical plasma jet which generates a multitude of different reactive oxygen and nitrogen species relevant in biological systems. Polarization status and oxidants had a profound impact on melanoma cell viability, and we identified various cytokines and chemokines important in that context. This study illustrates the importance and potential of the redox environment in tumor immune control.

P199 | Targeting melanoma cells at ribosomal biogenesis: inhibition of telomerase and RNA-polymerase I as a new therapeutic approach

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The enzyme telomerase sustains the telomeric ends of the chromosomes and thus allows indefinite growth of telomerase positive tumor cells. Additionally, telomerase stimulates cellular growth by enhancing the key step of ribosomal biogenesis, transcription of rDNA by RNA-polymerase I. 90% of all tumors activate telomerase and thus acquire immortality and a growth advantage. We hypothesize that a double treatment against telomerase and RNA-polymerase I transcription may impair ribosomal biogenesis and drive tumor cells into apoptosis. We treated HCT-116 cells with three telomerase inhibitors in combination with an inhibitor of RNA-polymerase I. The telomerase inhibition in our model was confirmed with TRAP-assay. The expression of the primary transcript of RNA-polymerase I, the 47S-precursor rRNA, was analyzed by RT-PCR. It could be observed, that the telomerase

inhibitors reduced the 47S-level to 50% as well as the inhibitor of ribosomal biogenesis. The combined treatment of telomerase inhibitors and RNA polymerase I inhibitor further suppressed the expression of the 47S-precursor rRNA. The analysis of cell growth and apoptosis by cell counting detected a growth inhibition by single and double treatment, whereas only the combination treatment induced apoptosis. These observations were further confirmed by Western blot analysis. It could be observed that p53 was stabilized in cells treated with inhibitors of telomerase and of ribosomal biogenesis.

Our observations indicate that inhibition of ribosomal biogenesis via telomerase and RNA-polymerase I transcription can effectively and specific kill cancer cells. For further analysis, melanoma cells are used. First results in the A-375 cell model indicate that the cells are effectively killed by inhibition of telomerase and RNA polymerase I. In addition, we want to analyze cell viability, senescence and apoptosis of the melanoma cells as well as of fibroblasts, keratinocytes and melanocytes as controls cells.

P200 (OP04/05) | RNA-Seq analysis of benign melanocytic nevi and primary melanomas

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Recent studies analysing hundreds of samples to characterize the melanoma mutational landscape using high-throughput sequencing technologies have supported the pathogenic role of the RAS/RAF/MAPK and PI3K/Akt pathways in melanoma biology, but also identified a series of new recurrently mutated genes such as NF1, GRM3, RAC1 and PREX2. However, a detailed analysis of downstream gene expression patterns and transcript variants contributing to early melanoma development is still missing. Here, a comprehensive RNA-Seq analysis of laser-microdissected benign melanocytic nevi (n=23) and primary melanomas (n=57) was performed, many of which were part of matched pairs (n=17). Unsupervised clustering of the transcriptomes clearly distinguished benign nevi from primary melanomas with more than 600 coding genes showing differential expression. Furthermore, a set of more than 350 non-coding RNAs showed differential expression. Among top upregulated genes in melanoma were MMP1, MMP8, MAGEA3, IL8, HOXD13, KIF23, CENPF, and PRAME, while KCNT2, ADAMTS19, and CASP12 showed significant downregulation. Further analysis showed significant enrichment of genes involved in the functional processes of mitotic nuclear division, immune response, locomotion, cell chemotaxis and defense response, some of which represent well-known molecular mechanisms active during development of different malignant tumors. Analysis by use of the protein interaction data base STRING identified three major networks involving cell cycle molecules such as AURKA, CDC20, and CENPA; chemokines/cytokines such as interleukin 8, CXCL10, and CXCL5; and transcription factors such SOX2, EYA4, and POUF1. Analysis of gene fusions revealed recurrent fusions involving a small set of genes. Some of these have been validated in independent sample sets. Taken together, the present melanoma transcriptome analysis by RNA-Seq of microdissected tissues identified genes and gene signatures that may contribute to early melanoma development.

P201 | Response patterns to MEK inhibition, but not NRAS mutation status predict the efficacy of combined MEK/CDK4,6 targeting in melanoma

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Treatment of NRAS mutant melanoma is challenging. Current targeted treatment modalities focus on the pharmacologic inhibition of signaling members downstream of NRAS. The discovery that co-targeting of MEK and CDK4,6 has antitumor activity created excitement for patients and clinicians; however, first clinical results have not met preclinical expectations.

In this study we investigate the response patterns of NRAS mutant melanoma cells in vitro and in vivo when challenged with inhibitors of MEK, CDK4,6 and the combination of both. Data revealed, that in vitro growth response patterns of cells treated with the MEK/CDK4,6 combination can be used to predict the in vivo efficacy of MEK/CDK4,6 co-targeting in a xenograft model of NRAS mutant melanoma. In addition, signaling changes after single MEK inhibition also correlated with the response to the MEK/CDK4,6 combination: Cells displaying activation of the cell cycle pathway after MEK inhibition evidenced by elevated pRb levels, showed more effective growth reduction with MEK/CDK4,6 co-targeting compared to single MEK inhibitor treatment. In contrast, MEK/CDK4,6 and single MEK inhibitor treatment were equally effective in cells that responded with unchanged or decreased protein levels of pRb after single MEK inhibition.

Cells sensitive to MEK/CDK4,6 co-targeting, defined by these criteria, showed a significant reduction of tumor size and robust induction of apoptosis in vivo. Strikingly, this pattern is not limited to NRAS mutant cells, but can be applied to BRAF mutant cells and cells that are “wild type” for these mutations.

Results of this study reveal that the MEK/CDK4,6 combination effectively reduces growth of a subset of NRAS mutant melanoma cells. However, findings suggest that mutant NRAS alone is insufficient to predict effective growth reduction with MEK/CDK4 targeting. Further, MEK/CDK4,6 has antitumor potential in cells with genetic driving alterations other than NRAS mutations, and might thus offer a new treatment strategy for an extended cohort of patients with malignant melanoma. Results suggest that the efficacy of the MEK/CDK4,6 combination can be predicted by in vitro viability assays and by the changes of pRb levels of cells after single MEK inhibition.

P202 | Evaluation of the radiation-induced stress response in a human full thickness skin model

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There are many publications dealing with the effects of X-radiation on molecular level using monolayer cell cultures, but until now only little is known about the radiation-induced stress response of cells imbedded in a tissue environment. Since it is conceivable that the cellular response differs due to the surrounding environment eg, extracellular matrix and cellular crosstalk the aim of this study was to evaluate our human full thickness skin model (FTSM) for radiation research. Based on a commercially available collagen/elastin-matrix (MatriDerm®) a FTSM was established by integrating primary human fibroblasts and keratinocytes. To test the usability of FTSMs for investigations of radiation-induced molecular mechanisms the capacity to repair radiation-induced DNA double strand breaks (DSB), the induction of apoptosis and checkpoint activation was examined. Immunostaining of 53BP1 and yH2AX showed that both cell types have the ability to mark and repair radiation-induced DSBs. Via TUNEL-analysis we demonstrated that X-radiation induced apoptosis in the fibroblasts of the dermal part. During the first 24 hours BrdU-uptake as well as the number of Ki-67 positive cells were reduced in epidermal keratinocytes. Both cell types integrated in the FTSM show radiation-induced stress response with little deviations to published effects in monolayer cells. Therefore, this tissue-like culture is suitable for investigating molecular processes in response to ionizing radiation in a tissue environment.

P203 | G-protein coupled receptor GPR120 influences melanoma cell proliferation, migration and cell cycle

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Omega-3 and Omega-6 free fatty acids are widely discussed in cancer research. Interestingly, these fatty acids can act directly as signaling molecules in cells via free fatty acid receptors (FFAR), which are members of the G-protein coupled receptor family (GPR).

Immunohistochemical expression analysis in paraffin embedded tissue samples revealed significant higher expression of the G-protein coupled receptor 120 (GPR120) in primary melanoma and melanoma metastases compared to nevi.

This observation suggests a functional relevance of GPR120 expression in the pathogenesis of melanoma. In order to test this idea the melanoma cell line A375, which expresses endogenously high levels of GPR120, was utilized. By applying the CRISPR/Cas technology a stable GPR120 knockout melanoma cell line was established. The knockout was verified on DNA level by TOPO-TA cloning and further sequencing. Functionally, the GPR120 knockout was confirmed by a FACS-based Fluoforte calcium release assay which showed a distinct lower calcium release in the knockout clone after treatment with a specific GPR120 agonist.

In cell proliferation analysis GPR120 knockout cells showed a significant lower proliferation-rate compared to the wild-type. Cell-cycle analysis of the knockout cells revealed a marked increase of the sub-G0 phase indicating higher apoptosis in this cell line. Moreover, GPR120 knockout cells showed reduced cell migration compared to the wild type as tested in a scratch assay.

Our first results point to a functional role of GPR120 in melanoma tumor biology. Therefore, it could be speculated that targeting this receptor may provide a novel therapeutic option. Further investigations are necessary to evaluate the specific mechanisms resulting in the observed changes in cell proliferation, apoptosis and cell migration.

P204 | Sensitization of melanoma cells for TRAIL-induced apoptosis by an indirubin derivative—a decisive role of reactive oxygen species (ROS)

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Melanoma remained a deadly disease despite recent developments of effective therapies as selective BRAF inhibitors and immune checkpoint inhibitors. The identification of even further strategies and combinations thus appears as important. Both the death ligand TRAIL (TNF-related apoptosis-inducing ligand) and derivatives of indirubin as kinase inhibitors appear as such promising strategies.

Here, we used a new indirubin derivative (DKP-073), which significantly induced apoptosis in melanoma cell lines. Apoptosis was strongly enhanced up to 30% at 24 hours in the combination with

TRAIL, as determined by cell cycle analyses. This was accompanied by almost complete loss of cell viability upon combination treatment (>90%, at 24 hours), as determined by calcein staining. In contrast, cellular release of lactate dehydrogenase (LDH), indicative for direct cytotoxicity, was neglectable. Also, the effects of TRAIL and indirubin alone on cell viability were much less pronounced.

Proapoptotic caspase activation (processing) was seen at 24 hours of treatment with indirubin alone and was strongly enhanced by the combination with TRAIL. Indirubin/TRAIL resulted in loss of mitochondrial membrane potential at 4 hours, indicative for intrinsic proapoptotic pathways. By Western blotting, we found upregulation of TRAIL receptor 2 (DR5) and downregulation of antiapoptotic XIAP, seen already at 6 hours of treatment with indirubin alone.

As an important clue for explaining the proapoptotic enhancement by indirubin, we found a massive increase of reactive oxygen species (ROS), which appeared already at 0.5 hour of treatment with indirubin alone. The critical role of ROS was demonstrated by the antioxidant N-acetyl cysteine (NAC). Thus, pre-treatment of melanoma cells with NAC not only abolished ROS production by indirubin, but almost completely blocked apoptosis and loss of cell viability by indirubin/TRAIL combination treatment. As concerning the mechanisms, ROS scavenging by NAC also prevented caspase activation, loss of mitochondrial membrane, XIAP downregulation and upregulation of DR5. These findings unravel the particular mechanism of indirubin in melanoma cells. Furthermore, they are suggestive for new, ROS-based therapeutic strategies for melanoma.

P205 | Critical role of reactive oxygen species (ROS) for synergistic enhancement of apoptosis by vemurafenib and the potassium channel inhibitor TRAM-34 in melanoma cells

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Inhibition of MAP kinases by BRAF inhibitors as vemurafenib and dabrafenib, has developed to a key therapy of BRAF-mutated melanoma. However, tumor relapse and therapy resistance remained as major problems, which may be addressed by combinations with inhibitors for other pathways. We identified here the potassium channel inhibitor TRAM-34 as highly effective in combination with vemurafenib. Thus, apoptosis was significantly enhanced and cell viability was decreased, whereas cytotoxicity was less affected. The combination vemurafenib/TRAM-34 also triggered apoptosis in vemurafenib-resistant cells, suggesting that acquired resistance may be overcome. Vemurafenib decreased ERK phosphorylation, suppressed antiapoptotic Mcl-1 and enhanced proapoptotic Puma. The combination resulted in activation of proapoptotic pathways as caspase-3 and loss of mitochondrial membrane potential. Indicative for a special mechanism in vemurafenib-induced apoptosis, we found strong enhancement of intracellular ROS levels already at 1 hour of treatment. The critical role of ROS was demonstrated by the antioxidant vitamin E (alpha-tocopherol), which decreased intracellular ROS as well as apoptosis. Also caspase activation and loss of mitochondrial membrane potential were suppressed, proving ROS as an upstream effect. Thus, ROS represents an initial and independent apoptosis pathway that is of particular importance for vemurafenib and its combination with TRAM-34 in melanoma cells.

P206 (OP05/01) | Presence and influence of neutrophil extracellular traps in malignant melanoma

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Background: The survival rates of patients with ulcerated melanoma are worse compared to patients with non-ulcerated melanoma (5-year survival rate Stage I: 55% vs 80%, Stage II: 12% vs 53%). Ulcerated melanoma regularly shows infiltration with intratumoral neutrophils that are associated with poor relapse-free survival, melanoma-specific survival and overall survival. The mechanism is only partially understood.

Neutrophil extracellular traps (NETs) are chromatin structures loaded with lysosomal proteins that are released by neutrophils for defense against pathogens and have been described to support tumor progression in Ewing sarcoma. Therefore we hypothesized that NETs are released in ulcerated melanoma and facilitate tumor progression.

Methods: Formalin-fixed, paraffin-embedded primary melanomas of 40 patients, 33 ulcerated and 7 non-ulcerated melanomas of metastasized and non-metastasized patients, were screened for infiltration of neutrophils by HE and CD15 staining. Extent of ulceration in tumors was measured after staining for panmel (melanoma marker) and pankeratin (epithelial marker). In order to detect NETs melanomas were stained for citrullinated histone H3 (H3Cit), myeloperoxidase (MPO), elastase and DNA. To investigate mechanisms of melanoma cell and NET interaction in vitro a static adhesion assay, XTT-assay, Transwell-Matrigel migration assay and immunofluorescence staining for caspase 3 as a marker for apoptosis were performed.

Results: In ulcerated and necrotic areas of melanomas with strong neutrophil infiltration NETs were present. The amount of NETs was dependent on the extent of melanoma ulceration (median ulceration 34%; Range: 2%-93%). In vitro assays revealed that melanoma cells adhere to NETs and that tumor cell migration is inhibited by NETs. In addition, co-culturing NETs and melanoma cells had a cytotoxic effect on melanoma cells, possibly by inducing apoptosis.

Conclusion: We have shown the presence of NETs in ulcerated and necrotic melanoma for the first time. Seeing that the deposition of NETs is limited to ulcerated and necrotic areas, we hypothesize the infiltration of neutrophils is a part of wound healing. We furthermore assume the production of NETs as a defense mechanism against microorganisms rather than a specific melanoma cell induced

immunoediting. Since NETs were found in proximity to tumor cells, we analyzed their influence on melanoma cell lines. In contrary to the initial hypothesis that neutrophils could promote tumor progression we show in vitro that NETs have an antineoplastic effect. NETs inhibit tumor cell migration eventually because of their web-like structure. Further, NETs have a cytotoxic effect on melanoma cells which might be due to their lysosomal proteins. It needs to be further elucidated why ulcerated, neutrophil rich melanomas are more aggressive than non-ulcerated melanomas despite the antineoplastic effect of NETs.

P207 | Ineffective antibody-dependent cellular cytotoxicity in patients with late stage cutaneous T cell lymphoma

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Introduction: Targeted therapies and immune modulators are currently changing our understanding for the treatment of solid tumors, and promise to open a new perspective in the management of cutaneous T-cell lymphoma (CTCL) as well. The mechanisms of action of therapeutic antibodies in vivo is not fully elucidated in all cases, antibody-dependent cellular cytotoxicity (ADCC) mediated by natural killer (NK) cells often being presumed to be a key mode of action. However, since progressive impairment of cellular immunity is a hallmark of CTCL, we questioned the fact that patients with late stage CTCL will still be in a possession of fully functional ADCC.

Objective: To investigate the mechanism of ADCC in CTCL patients.

Materials and methods: NK cells were isolated from patients with MF stage I-IV, Sézary syndrome (SS) patients and healthy individuals. An aCella-TOX GAPDH assay was used to detect the amount of endogenous glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the level of ADCC in each individual patient.

Results: In vitro ADCC in patients with MF stage I was comparable to that of healthy individuals, but severely abrogated in all MF Stage IV and SS patients included in the study. The percentage of NK cells in the blood of CTCL patients was within normal limits. Trogocytosis, a mechanism of cellular communication that can hamper ADCC by cleaving the surface of the tumor cells from the targeted molecule, seemed not to play an essential role in CTCL. However, overexpression of MHC I on the malignant tumor cells in CTCL was important factor in helping tumor cells escape NK-cell activity and MHC I blockade could restore impaired ADCC.

Conclusions: Impaired ADCC may pose some problems when choosing a targeted drug therapy for the treatment of late stage CTCL. Understanding of the immunological mechanisms behind it will help improve NK cell activity in CTCL patients and overcome resistance to treatment.

P208 | Thymoma-associated autoimmune syndrome

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Thymoma-associated multiorgan autoimmunity (TAMA) is a rare condition characterized clinically by mucosal and skin lesions resembling graft-versus-host disease (GVHD). The pathogenesis of TAMA is presumably linked to an altered immune surveillance with deficient down-regulation and/or depletion of self-reactive T cells linked to tumors of the thymus which is critical for the regulation of central immune tolerance against self-antigens. We here present a patient with relapsing thymoma who developed several cutaneous and extracutaneous autoimmune disorders. Initially, the 51-year-old female patient developed myasthenia gravis (MG) and was later diagnosed with thymoma which was removed 7 years ago. Six years later, she developed pemphigus foliaceus (PF) with anti-desmoglein 1 IgG autoantibodies which eventually disappeared upon treatment with rituximab. Seven months later, the patient developed erythroderma with a lichenoid inflammatory skin infiltrate characteristic of GVHD. She was then diagnosed with a local relapse of thymoma which was fully excised leading a gradual regression of the skin lesions. The patient has now developed lichenoid mucosal lesions with residual lichenoid plaques on the thighs. Noteworthy, MG had been active during the entire observation period. Presumably, relapsing thymoma led to an additional loss of tolerance against self-antigens of the skin leading to PF and GVHD, respectively. These phenomena were temporary and improved upon treatment of the thymoma while the clinical activity MG remained largely unaffected.

P209 (OP02/04) | Insulin resistance as a pathomechanism in malignant melanoma?

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Malignant melanoma is one of the most aggressive cancers and despite a growing number of promising therapeutic approaches, the prognosis remains poor for most patients. There is evidence that the risk for several cancer types like pancreatic, hepatic, colorectal and breast cancer is increased in diabetic patients and that molecular insulin resistance may represent a pathomechanism in carcinogenesis. In malignant melanoma this correlation is still unclear. However, first indications of a potential association between obesity and insulin resistance as an independent risk factor have been pointed out.

Thus, we investigated in situ as well as in vitro, whether molecular insulin resistance contributes to carcinogenic alterations in melanoma. Immunhistochemical staining of melanoma specimen of different tumor stages revealed signs of molecular insulin resistance as measured by inhibitory phosphorylation events of the insulin receptor substrate (IRS-1).

Simulating hyperinsulinemia in vitro by chronic exposure of melanoma cell lines to insulin, made the PI3-K/Akt pathway resistant to further insulin stimulation, which is characteristic of molecular insulin resistance. Blocking mTOR (mechanistic target of rapamycin) or MAPK with either chemical inhibitors or siRNA-mediated knockdown restored insulin sensitivity suggesting that oncogenic hyperactivation of these kinases contributes to molecular insulin resistance and could represent a carcinogenic pathomechanism. When investigating the physiological effect of insulin resistance, we found no effect on cell proliferation or cell migration in scratch assays. Interestingly, hyperinsulinemia increased the expression of adhesion molecules such as I-CAM, Mel-CAM or αVβ3 integrin, which was prevented when mTOR signaling was inhibited with rapamycin. As increased expression of adhesion molecules could be indicative of an increased metastatic potential, we analyzed the migration of insulin resistant cells in a transwell assay. We found that under conditions of insulin resistance, melanoma cells show significantly increased migration.

Thus, insulin resistance of melanoma cells in hyperinsulinemic patients could contribute to the aggressiveness of malignant melanoma by enhancing the metastatic potential of tumor cells. In summary, the results of this work not only contribute to a better understanding of the pathomechanisms in malignant melanoma, but suggest to investigate whether restoration of insulin sensitivity could be beneficial for the success of conventional anti-tumorigenic strategies.

P210 | Dual role of aPKCι/λ in skin carcinogenesis

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Altered polarity and tissue architecture is a hallmark of cancer and metastasis. The atypical kinas C lambda (aPKCλ)a central regulator of cell polarity, is considered a bonafide tumor promoter as its loss either prevents or strongly inhibits tumor formation in eg, mouse models for colon or lung carcinoma. In accordance, aPKCι/λ is overexpressed in many human carcinomas and this overexpression is associated with metastasis and a poor prognosis. In order to study the role of aPKCι/λ in non-melanoma skin carcinogenesis we first performed two-step DMBA-TPA skin carcinogenesis in control and epidermal aPKCλ knockout mice. Here we found that loss of aPKCλ-inhibited Ras-mediated papilloma formation, likely due to a decrease in survival and growth signaling resulting in increased apoptosis and reduced proliferation. We then asked whether an increase in aPKCλ activity would promote Ras-induced papilloma induction. To this end we generated mice with epidermis specific expression of a membrane-targeted version of aPKCλ(aPKCλcaax), previously shown to drive overgrowth and tumor formation in *Drosophila*. Despite opposite phenotypes in epidermal homeostasis compared to loss of aPKCλ, aPKCλcaax also inhibited papilloma formation, albeit that tumor cells ultimately shut of expression of this transgene. Finally, to more faithfully mimic human squamous cell carcinoma, mice with an epidermal specific deletion of aPKCι/λ were crossed to mice with an epidermal specific deletion of p53, who develop squamous cell carcinomas at a median age of 330 days. Even though these latter mice do not show an obvious developmental phenotype, the aPKCλ/p53epi−/−mice are more fragile than aPKCλepi−/− alone, suggesting that additional loss of p53 aggravates skin barrier dysfunction induced by loss of p53. To our surprise, aPKCλ/p53epi−/− show a strongly hyperthickened epidermis and develop moderately differentiated squamous cell carcinomas starting at an age of 90 days whereas the epidermis p53epi−/− mice show only moderate signs of dysplasia, but no macroscopic phenotype. Brdu incorporation assays revealed a strong increase in proliferation in aPKCλ/p53epi−/− mice compared to the single knockouts, which is first observed at P21 whereas apoptosis is only minimally increased. Together, our results unravel a dual role for aPKCλ in non-melanoma skin tumor models and surprisingly indicate that aPKCλ is not only a tumor promoter but, depending on the context, can also serve as a tumor suppressor.

P211 | Cell death and senescent cancer cell clearance

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Cellular senescence is an intrinsic proliferation stop important for organ development, homeostasis and disease. Cellular senescence also prevents the transformation of pre-malignant lesions into malignant cancer and can even arrest fully developed cancers. This proliferative stop can be induced by exogenous immune cell-derived signals. The TH1 cytokines IFN-γ and TNF can hinder the proliferation of B16 melanoma cells in vitro and induce senescence in murine β-cancer cells in vitro and in vivo. If cancers are driven into senescence the remaining senescent cancer cells bear the risk of promoting cancer. Therefore, clearance of senescent cancer cells is an important goal. Here, we analyzed the vulnerabilities of senescent β-cancer cells and ask whether cell death of senescent cancer cells is needed for cancer cell clearance. We first show that immature bone-marrow derived macrophages (bmMΦ) phagocytize apoptotic cancer cells while senescent cancer cells are resistant to phagocytosis. We therefore studied whether or not senescent cancer cells were susceptible to undergo apoptosis. Surprisingly we found that senescent β-cancer cells were more susceptible to secondary apoptosis induced by the kinase inhibitor staurosporine or the TNF related apoptosis inducing ligand (TRAIL)

than normal cancer cells. These secondary apoptotic cells were efficiently phagocytized by bmMΦ. β-cancer cells driven into senescence via IFNy and TNF show a highly inflammatory SASP consisting of various chemokines, eg, CXCL12 and CCL5. These two chemokines are known to attract and polarize surrounding macrophages. Bone-marrow derived macrophages (bmMΦ) show an increased migratory capacity and are polarized to a M2-like phenotype when challenged with the supernatant of cytokine-induced senescence cells. M2-like macrophages were characterized by a lack of iNOS expression and at the same time increased arginase1 levels. Polarized bmMΦ showed a decreased phagocytic ability in comparison to naïve bmMΦ. As a consequence, these led to a failure of phagocytosis of senescent or non-senescent β-cancer cells in vitro, even though senescent β-cancer cells downregulate CD47, a surface marker preventing the recognition of cells by the immune system, and attract bmMΦ via the chemokines secreted by the SASP. In summary, our data unraveled a potential pathway of senescence cell clearance by secondary apoptosis.

P212 | Damage-associated molecular pattern molecules as novel predictive markers in melanoma treatment with immune checkpoint inhibitors

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Immune checkpoint-inhibition using monocolonal antibodies targeting PD-1 is a revolutionary therapeutic approach in metastatic melanoma patients and is characterized by a significant extension of overall survival and long-term responses.

Recently, serum levels of damage-associated molecular pattern molecules (DAMP) such as S100A8/A9 and HMGB1 have been shown as predictive biomarkers for immune checkpoint-inhibitor ipilimumab in melanoma patients. Elevation of both serum markers correlated significantly with a lacking response to treatment.

Here, we present a retrospective analysis of 31 melanoma stage IV patients treated with the anti-PD-1 antibody pembrolizumab using serum samples before and during treatment for serum level analysis of S100A8/A9 and HMGB1 by specific enzyme-linked immunosorbent assays. Treatment response was measured after 3, 6, and 9 months by radiological immune-related response criteria. Patients lacking a radiological response to treatment demonstrated elevated serum levels of both S100A8/A9 and HMGB1 after the first two treatment cycles compared to baseline levels. In contrast, treatment responders were characterized by low and decreasing levels of both DAMPs under pembrolizumab treatment.

In summary, our observations point towards a similar marker ability of serum S100A8/A9 and HMGB1 as predictive biomarkers of a poor response to anti-PD-1 as compared to ipilimumab treatment. Both markers will aid in the identification of patients that may experience a long-term response to immune checkpoint-inhibition. Moreover, our data shed light on the complex biology of (non)response to novel immune-checkpoint inhibitors.

P213 | Tumor-homing eosinophils predict the clinical course of malignant melanoma

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The interaction of the patient's immune system with melanoma impacts on the clinical outcome and might provide important implications for the identification of prognostic markers. The specific immune reaction is represented by immune cell infiltrates.

Here, we systematically analyzed the presence and localization of tumor-infiltrating immune cells on tissue-microarrays displaying 59 primary melanoma, 70 corresponding metastases as well as in 41 associated benign nevi and evaluated their clinicopathological impact and using the Kaplan-Meier method and Cox proportional hazards model, and Mann-Whitney-*U* or Kruskal-Wallis test. Immune cells were detected using immunohistochemistry and specific antibodies.

Higher levels of activated eosinophils as well as tumor-infiltrating T lymphocytes, and T-memory cells were significantly associated with longer progression-free (PFS) as well as overall survival (OS) whereas higher levels of tumor-infiltrating neutrophils were significantly associated with shorter PFS and OS.

Eosinophils as well as tumour-infiltrating T cells, memory T cells, and neutrophils are independent prognostic tissue markers that might be central for elucidating the specific immune cells-melanoma cell interaction.

P214 | Peripheral myelin protein 2 is a target gene of the transcription factor SOX10 in melanoma and affects tumor cell invasion

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The transcription factor SRY (sex determining region Y)-box10 (SOX10) plays a key role in the development of melanocytes and glial cells from neural crest precursors. There is growing evidence that SOX10 is involved in melanoma initiation, proliferation, invasion,

and survival. We have identified SOX10 as a regulator of melanoma cell invasion by its target gene melanoma inhibitory activity (MIA). However, specific mediators which impart the oncogenic properties of SOX10 in melanoma remain widely unknown.

To further identify potential target genes of SOX10, we performed RNA sequencing to analyze genome-wide expression alterations after ectopic expression of SOX10 in the metastatic 1205Lu melanoma cell line. Among nine genes differentially regulated by SOX10, only peripheral myelin protein 2 (PMP2) was found upregulated in 1205Lu and two other melanoma cell lines. The fatty acid binding protein PMP2 is one of the most abundant myelin proteins in the peripheral nervous system. It has not been described in melanoma before. We found PMP2 to be downregulated by SOX10 inhibition and detected mRNA expression in melanocytes and melanoma cell lines but not in fibroblasts. However, protein expression was restricted to a few melanoma cell lines and was absent in melanocytes. Direct binding of SOX10 to the PMP2 promoter was shown by chromatin immunoprecipitation and electrophoretic shift assays. Two of three in silico predicted SOX10-binding sites within the PMP2 promoter region can be activated by SOX10 as shown by reporter assays. Previous studies have shown that SOX10 together with the transcription factor early growth response 2 (EGR2) regulate the expression of myelin proteins in Schwann cells. We performed co-inhibition studies that also suggest a co-regulation of PMP2 expression by SOX10 and EGR2 in melanoma cells. Inhibition of PMP2 in PMP2-positive but not in PMP2-negative melanoma cell lines reduced cell number, morphology, and cell viability about 3 days after siRNA transfection. However, cell viability was not increased upon PMP2 overexpression. Interestingly, stable PMP2 expression in a PMP2-negative melanoma cell line significantly increased invasion in two- and three-dimensional assays. Overexpression of a PMP2 mutant isoform, which affects PMP2 association to the cell membrane, reduced the invasion capacity compared to the wild-type isoform.

In conclusion, PMP2 is a newly identified target gene of SOX10. PMP2 expression has a positive effect on melanoma cell invasion although its general expression is restricted to a subset of melanoma cell lines which might be related to tumor heterogeneity.

P215 (OP02/05) | Inhibition of FGF receptors for effective treatment of BRAF- and BRAF/MEK inhibitor-resistant melanoma cells

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Treatment of metastatic melanoma has evolved substantially within the last decade. Due to the frequent occurrence of acquired resistance to BRAF inhibitors (BRAFi), current therapeutic strategies now concentrate on combinatorial targeted treatment to block reactivation of MAPK signaling and other resistance pathways. The combination of BRAFi and MEK inhibitors (MEKi) has achieved higher response rates and prolonged survival compared to BRAFi monotherapy and has led to their clinical approval. However, therapy resistance is still only delayed but not abrogated.

In this study, we generated three different inhibitor-resistant melanoma cell lines by continuous treatment with the BRAFi vemurafenib or the combination vemurafenib plus the MEKi selumetinib. We then investigated cell viability in untreated parental cells, BRAFi-, and BRAFi/MEKi-resistant cells upon exposure to the PI3K inhibitor BKM-120, ERK inhibitor GDC-0994, and pan fibroblast growth factor receptor (FGFR) inhibitor BGJ-398 alone or in combination with vemurafenib and selumetinib. Different effects were observed in different cell lines. The highest rate of cell death was found for the triple combination of either BKM-120 or BGJ-398 with vemurafenib and selumetinib.

In one cell line a striking reduction of cell viability was observed with BGJ-398 treatment alone, while BRAFi- and BRAFi/MEKi-resistant cells were more sensitive than parental cells. This observation went along with a higher increase of apoptosis in the resistant compared to the parental cells. In a three-dimensional spheroid cell culture model resistant cells showed stronger invasion than parental cells. However, upon treatment with BGJ-398 alone or in combination with vemurafenib and selumetinib, resistant cell lines almost completely lost their invasive capacity and spheroids considerably decreased in size.

On molecular level we found increased expression of FGFR1, FGFR2, FGFR3, and FGFR4 in the resistant compared to the parental cells. Furthermore, resistant cells showed enhanced expression and secretion of FGF7. A human protein-profiler phosphokinase array revealed enhanced activation of the MAP kinases ERK and p38, the PI3K target AKT, and enhanced canonical Wnt signaling. Other resistant cell lines that did not respond to BGJ-398 treatment differed in their downstream signaling. How these molecular patterns influence the response to FGF receptor inhibition is currently under investigation.

In conclusion, our data provide evidence for a strong heterogeneity in acquired resistance in vitro and suggest that inhibition of FGF signaling inhibition might be a promising compound for future combinatory treatment strategies against BRAFi- and BRAFi/MEKi-resistant melanomas.

P216 | LRIG2—friend or foe in skin tumorigenesis

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The ERBB receptors (ERBB1-4) are important regulators in the skin regarding development, homeostasis and tumorigenesis. A disturbed ERBB signaling cascade can result in positive and negative feedback loops, depending on the ligand. Recently, the leucine-rich repeats and immunoglobulin-like domains (LRIG) family attracted attention because of their influence on these feedback mechanisms. The protein

family consists of three transmembrane proteins (LRIG1-3) with different functions in different tissues. Whereas LRIG1 and LRIG3 seems to have similar function, LRIG2 is more unique and may oppose the function of its family members. It was shown that an increased LRIG2 expression in patients with astrocytic tumors is related to good prognosis. Contrary, a high expression level of LRIG2 in samples of patients with squamous cell carcinoma of the uterine cervix or non-small cell lung cancer served as a prognostic marker for poor survival. LRIG2 is widely expressed in human skin but nothing is known about its function during homeostasis or tumorigenesis in the skin. Therefore, we generated a doxycycline-inducible, skin-specific (keratin-5-promoter) transgenic mouse line overexpressing LRIG2 using the TET-OFF system. In our model we investigated the effect of LRIG2 overexpression on ERBB receptors in a UVB-irradiation experiment. The back skin of HA-LRIG2 TG mice and control animals was irradiated twice with UVB light (200 mJ/cm^2) and western blot analysis of the irradiated skin revealed a decreased activation of ERBB receptors in transgenic animals. Additionally, we investigated tumor development and progression in a multi-stage chemical skin carcinogenesis. In this experiment we observed a reduced papilloma burden in HALRIG2 TG mice and a decreased mean papilloma size. Although the papilloma incidence was not altered between both groups.

The present data indicate that LRIG2 signaling reduce papilloma growth during multi-stage chemical carcinogenesis in mice and imply that this new LRIG2 gain-of-function mouse model will contribute to a better understanding of the impact of LRIG2 during skin carcinogenesis.

P217 | Combination with γ-secretase inhibitor prolongs treatment efficacy of BRAF inhibitor in BRAF-mutated melanoma cells

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Oncogenic triggering of the MAPK pathway in melanocytes results in senescence, and senescence escape is considered as one critical step for melanocytic transformation. In melanoma, induction of a senescent-like state by BRAF-inhibitors (BRAFi) in a fraction of treated cells—instead of killing—contributes to repression of tumor growth, but may also provide a source for relapse. Here, we demonstrate that NOTCH activation in melanocytes is not only growth-promoting but protecting these cells against oncogene-induced senescence. In turn, treatment of melanoma cells with an inhibitor of the NOTCH-activating enzyme γ-secretase led to induction of a senescent-like status in a fraction of the cells but achieved overall only a moderate inhibition of melanoma cell growth. However, combination of γ-secretase inhibitor (GSI) with BRAFi markedly increased treatment efficacy particularly in long-term culture. Moreover, even melanoma cells starting to regrow after continuous BRAFi treatment—the major problem of BRAFi therapy in patients—can still be affected by the combination treatment. Thus, combining GSI with BRAFi increases the therapeutic efficacy by, at least partially, prolonging the senescent-like state of treated cells.

P218 | Hyperactive NRAS downstream signaling induces specific transcriptome changes -esiRNA-based identification of new therapeutic targets in NRAS mutant melanoma identifies the noncoding RNA 7SL as a major proliferation enhancer

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Benign naevi and malignant melanomas both can have oncogenic mutations in the NRAS gene but naevi only rarely progress to cancer. Such NRAS mutations should lead to specific transcriptome changes. The knowledge of these changes can (i) identify new therapeutic targets and (ii) explain why some naevi have NRAS mutations but stay benign. Here, we introduce NRAS(Q61) mutant plasmids in a pool of human melanocytes to overactivate NRAS downstream pathways. We perform deep RNASeq and compare transcriptome changes in melanocytes with and without NRAS mutation. We list differentially expressed coding and noncoding transcripts by filtering our results with transcriptomes from 2 NRAS mutant melanoma cell lines and 89 NRAS mutant patient tumors. Next we use esiRNA (endoribonuclease-prepared siRNA) libraries to knock down these transcripts and perform proliferation assays to identify potential targets. For most promising candidates we perform further siRNA knockdowns, cell-based assays and take a closer look at their mechanistics.

Our approach identified 237 transcripts, of which 2 coding and 6 noncoding transcripts played an important role in the proliferation of NRAS mutant melanoma. The knockdown of each of these transcripts led to cell proliferation decreases of 30%-60%. We focused on the noncoding RNA RN7SL1 and identified its up-stream regulation (MAPK pathway) and its downstream effectors (p53). In conclusion, we identify new therapeutic targets which might be used in the battle against NRAS mutant melanoma.

P219 (OP01/03) | c-MET dependent neutrophil responses limit anti-tumoral T cell expansion and immunotherapy of cancer

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Background: Oncogenic signal transduction inhibitors and T-cell immunotherapies are currently revolutionizing cancer treatment. The HGF/c- MET signalling pathway is dysregulated in many solid cancers, but the therapeutic benefit of targeting oncogenic c-MET is limited to subgroups of patients with certain cancer types. HGF/c-MET signalling also participates in the regulation of immune responses. Therefore, we hypothesize that c-MET inhibitors could increase the efficacy and have a broader applicability in combination with immunotherapies.

Methods: To test this hypothesis we treated tumor-bearing mice a selective c-MET inhibitor (METi) alone or in combination with T-cell immunotherapies.

Results: We identified c-MET inhibition (METi) as an adjuvant treatment strategy to enhance the efficacy of T-cell immunotherapies in different cancer mouse models. This therapeutic benefit was irrespective of tumor cells' dependence on c-Met signalling, but instead due to impaired reactive neutrophil responses. We found out that HGF/-c-MET signalling promotes the egress of neutrophils from the bone marrow and the recruitment into T-cell inflamed tissues in response to cancer immunotherapies. Importantly, neutrophils acquired an immunosuppressive phenotype and thereby restrained anti-tumoral T-cell expansion, in particular in the tumor draining lymph node, and thus limited immunological tumor control. Pharmacological inhibition or genetic ablation of c-MET reduced the number of immunosuppressive neutrophils in the draining lymph node which led to an increased T-cell expansion and thereby improved tumor control.

Conclusion: Our results strongly support the rational that inhibition of c-MET increases the efficacy of cancer immunotherapies. As reactive neutrophil responses are also seen in patients, our findings provide scientific basis for adjuvant c-MET inhibitor treatment to improve immunotherapeutic approaches in a variety of cancer.

P220 | "Off-target" effects of tyrosine kinase inhibitors on human vitamin D3 metabolism

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Chronic myeloid leukemia (CML) is a myeloproliferative disease characterized by an upregulated activity of tyrosine kinase (TK) BCR-ABL1. TK inhibitors like imatinib (IMA) or nilotinib (NIL) inhibit this TK and have therefore changed the therapy of CML dramatically.

However, IMA and NIL exert "off target" side effects on bone metabolism in adult and pediatric patients. As vitamin D3 (VD3) is involved in the complex cycle of bone remodelling, we investigated the influence of IMA and NIL on the VD3 metabolism (i) in HaCaT cells and (ii) in cultured outer root sheath keratinocytes (KC) from hair follicles of TKI-treated children with CML.

Cells were incubated with 25 μmol/L 7-dehydrocholesterole and 1 μmol/L IMA or 1 μmol/L NIL, (corresponding to therapeutic plasma concentrations) and exposed to UVB irradiation Concomitantly, specific inhibitors were applied to analyze the inhibition of VD3 processing cytochrome P450 isoenzyme family by TKI. KC of TKI-treated children were cultured for 28 days. Calcidiol and calcitriol levels were determined quantitatively using ELISA technique after an incubation period of 24, 48, or 72 hours, respectively.

In vitro at the clinically effective concentration both TKIs tested significantly impaired production of calcidiol and calcitriol. Compared to TKI-untreated controls calcitriol levels were reduced by IMA and NIL to 50% and 10%, respectively. Additionally, interaction studies performed with inhibitors of P450 enzyme family (VID400 and ketoconazole) in the absence of TKI did not influence calcidiol levels (range: 90-110 ng/1 × 10^6 cells) while calcitriol levels were decreased by about 60%. IMA in the presence of VID400 increased calcidiol levels by 600% but did not influence calcitriol synthesis. Co-incubation of IMA and ketoconazole increased calcitriol levels by 200%.

Identical effects were detected when the described experiments were repeated using keratinocytes of TKI treated children and healthy subjects. Furthermore, compared to KC of healthy subjects, KC of TKI treated children revealed no differences in levels of calcidiol and calcitriol. However, it must be taken into consideration that the long culture time for generating KC might override changes induces by prior in vivo pretreatment. In conclusion, IMA and NIL interfere with the VD3 cascade due to their metabolization by CYP27B1 and might explain partly the disturbed bone metabolism.

P221 | Identification of unique molecular signatures of differentially cycling tumour cell subpopulations in a 3D melanoma model

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Microenvironment-driven intra-tumoral dynamic heterogeneity is a leading cause of drug resistance acquisition in melanoma. 3D spheroids generated from fluorescent ubiquitination-based cell cycle indicator (FUCCI)-transduced melanoma cell lines revealed two differentially cycling subpopulations within each spheroid: a central G1-arrested and a peripheral proliferating subpopulation. Confocal microscopy of sectioned spheroids showed that expression of the

Microphthalmia-associated transcription factor (MITF) exclusively co-localized with the peripheral cycling population. To elucidate the molecular mechanism behind this phenomenon, we isolated cells from each subpopulation by Hoechst dye diffusion and FACS and then validated them by their respective MITF expression pattern. RNA seq analysis of cells isolated from these two different subpopulations revealed that the melanocyte- and melanoma-specific isoforms of MITF (MITF-M, MITF-Mdel), several upstream and downstream effectors of MITF, DNA repair and cell cycle promoting genes were significantly downregulated in the central G1-arrested compared to the peripheral cycling subpopulation. Pathway enrichment analysis of the RNAseq data suggested that the PI3K-AKT pathway is downregulated and the non-canonical Wnt/β-catenin pathway is upregulated in the central G1-arrested compared to the peripheral cycling subpopulation. Our ongoing studies aim to decipher the PI3K-AKT and noncanonical Wnt/β-catenin pathway driven regulatory mechanism behind the differential expression pattern of MITF in these differentially cycling tumour subpopulations. In addition, we will also investigate the downstream effectors of MITF to understand how differential expression of MITF and its activity in these two subpopulations regulate their segregation within spheroids.

P222 | Tumor cell intrinsic TLR4 signaling contributes to pathogenesis of metastatic melanoma

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Metastatic melanoma is the most deadly type of skin cancer as it spreads quickly. Others and we have shown that Toll-like receptor 4 (TLR4) signaling in the tumor microenvironment is important for tumor cell survival and metastasis. In this study we report that TLR4 inhibition in a number of human melanoma cells sensitized to TNF induced killing, and this was TLR4/NFKB signaling dependent. In patients TLR4 expression was significantly associated with a shortened relapse free survival. In mice TLR4 over expression caused enhanced tumor growth and increased lung metastasis. In vitro, TLR4 over expression enhanced sensitivity to inflammatory stimuli. Concurrently, deletion of TLR4 on tumor cells decreased tumor growth and lung metastasis. Taken together, our findings suggest that inflammation induced melanoma cell adaptation, in a course with enhanced TLR4 expression on tumor cells, is partly a mechanism underling melanoma pathogenesis. Our study provides new insights for therapeutic intervention with TLR4 antagonists as a combinatorial treatment option for melanoma patients with a high risk for metastatic melanoma.

P223 | CD73 (NT5E) expression is a prognostic marker for primary melanoma patients and a potential predictive marker for anti-PD1 treatment

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Background: Immune checkpoint inhibitors (anti-PD1, anti-CTLA4) are currently revolutionizing the treatment of advanced tumor diseases, including melanoma. However, the therapeutic efficacy is hampered by various mechanisms of resistance eg, tumor cell plasticity and metabolic alterations that drive immune evasion. Increased expression of the ecto-5′-nucelotidase (CD73) has been linked to an altered adenosine metabolism. The immunosuppressive metabolite adenosine shapes the activity of a variety of cell types in the tumor microenvironment and promotes tumor progression. Preclinical studies highlight the modulation of CD73 expression and activity as a novel approach for cancer immunotherapy. The prognostic significance for melanoma patients and the predictive value of CD73 expressing melanoma cells for anti-PD1 therapy has not been investigated so far.

Methods: To investigate the prognostic significance of CD73 for melanoma progression, we analyzed CD73 expression by immunohistochemistry and clinicopathologic features in a representative cohort of 124 primary melanomas with known sentinel lymph node status and in 75 cutaneous melanoma metastases. CD73 expression has been investigated in a cohort of 27 melanoma patients (kindly provided by Paul Tumeh and Antoni Ribas, UCLA, USA) that underwent biopsies before and during anti-PD1 antibody treatment to evaluate the predictive value of CD73 expression for anti- PD1 treatment. A semi-quantitative scoring systems (0=no expression, 1=low, 2=intermediate, 3=high) has been applied for CD73 expression intensity on melanoma cells.

Results: CD73 expression on primary cutaneous melanoma cells was significantly positively correlated with tumor thickness (T classification; *P*=.0013), ulceration (*P*=.0013) and positive sentinel lymph node status (N classification; *P*=.002). Histologic subtype, Tumor-infiltrating lymphocytes and pigmentation (measured by HMB45 expression) did not show any correlation with CD73 expression intensity. Patient with no or low CD73 expressing melanoma cells had a longer progression-free and overall survival than patients with intermediate or high CD73 expression (median follow-up time: 5.2 years). CD73 expression in cutaneous melanoma metastasis is often associated with areas of necrosis. In the cohort of 27 patients under anti-PD1 blockade, 2 (7%) patients showed a complete regression (CR), 12 (44%) a partial response (PR), 7 (26%) stable (SD) and 6 (22%) progressive disease (PD) based on irRECIST. 7/21 (33%) of the responders (CR, PR and SD) and 0/6 of the nonresponders (PD) showed a down-regulation of CD73 expression on melanoma cells during anti-PD1 blockade.

Summary/Outlook: The association of CD73 expression on melanoma cells with tumor thickness, ulceration and sentinel lymph node metastases suggest that CD73 is a worse prognostic marker for primary melanoma patients. Down-regulation of CD73 expression on melanoma cells during anti-PD1 blockade might be a potential predictive marker for therapeutic response. In summary, these findings support the hypothesis that CD73 induction on melanoma cells increases the tumor promoting activities of extracellular adenosine. Thus, CD73 represents a promising novel biomarker and target to overcome anti-PD1 therapy resistance.

P224 | MITF regulates cell adhesion and subcompartment-specific distribution of differentially cycling melanoma cells

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Differential tumor cell behavior caused by environmental conditions, termed dynamic heterogeneity, is a prime source for drug resistance. We utilize real-time cell cycle imaging (FUCCI) to study melanoma heterogeneity. As distinct proliferative and invasive capabilities reflect variable drug sensitivities, identifying and characterizing these different responses is crucial to design effective therapies. Mouse xenograft tumors generated from cell lines with high microphthalmia-associated transcription factor (MITF) level displayed a homogeneous distribution of cycling cells throughout. In contrast, tumors generated from cell lines with low MITF levels were composed of clusters of cycling cells and clusters of G1-arrested cells. The proliferating areas were in close proximity to blood vessels, presumably characterized by oxygen/nutrient availability. Melanoma spheroids recapitulated the in vivo cycling behavior, considering that here oxygen and nutrients are supplied by diffusion. MITF was undetectable within the hypoxic G1-arrested spheroid core, indicating hypoxia-induced MITF downregulation. Finally modulation of MITF expression impacted spheroid density, with overexpression giving rise to less compacted structures and vice versa. We conclude that MITF protects from cell cycle arrest induced by oxygen/nutrient deprivation. We hypothesize that high MITF levels prevent cell cycle arrest by reducing the cell-intrinsic propensity to arrest in response to low oxygen/ nutrient and concurrently by allowing sufficient supply of oxygen/nutrients to cells. The latter may be achieved through decreased cell-cell/matrix adhesion resulting in the generation of looser tumors that allows more efficient oxygen/nutrient diffusion. These data outline how MITF-regulated dynamic heterogeneity could influence therapy efficiency, making MITF an important marker for drug design.

P225 | CXCL5 and neutrophils increase lymphatic metastasis in cutaneous melanoma

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Previous analysis of human and mouse melanoma chemokine profiles showed that high expression of CXCL5 is in accordance with a worse disease progression. To investigate the role of CXCL5 in a syngeneic melanoma model in more detail, we intradermally transplanted murine CXCL5 (LIX) overexpressing and control B16F1 and HCmel12 melanoma cells into C57BL/6J wt mice. The main known function of CXCL5 is the chemoattraction of neutrophils by binding to their receptor CXCR2.

Chemokine profiling of these CXCL5 overexpressing tumors vs controls, showed that changing the expression of this particular chemokine upregulates CXCL2, CXCR2 and CXCR4 but does not affect the expression pattern of other well-known pro-tumorigenic chemokines. Besides that, a positive correlation of other CXCR2 ligands, CXCL1, CXCL2 and CXCL8, in concert with CXCL5 was found in primary human melanoma samples in various publicly available databases.

CXCL5 overexpressing tumors compared to control tumors of both melanoma cell lines showed an increased frequency of lymph node metastasis. Analysis of intratumoral and peritumoral lymph and blood vessel densities in immunohistochemically stained tumor sections did not differ between the two groups, which gives CXCL5 and its recruited neutrophils more importance being the active key players, determining the metastatic route. Characterization of tumor associated neutrophils (TANs) using flow cytometry showed a distinct phenotype compared to neutrophils of blood and bone marrow.

In future experiments, treatment with a neutrophil depletion antibody of tumor bearing mice will differentiate between the effect of CXCL5 alone or in combination with the neutrophils on lymph node metastasis. In another experiment, tumor bearing mice will be treated with Poly(I:C) to unravel interactions between neutrophils and T-cells, an important encounter for further immunotherapies.

P226 | Interplay between aryl hydrocarbon receptor signaling and inflammatory responses in melanoma

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Expression and activation of the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor known to sense and to

orchestrate cellular responses to various environmental stimuli, has been shown to play a role in a set of infectious and malignant diseases as well as in peripheral immunity. Recent studies provide a link between AhR and the physiological regulation of skin pigmentation as well as several pathological skin processes including carcinogenesis and inflammation. However, the molecular role of AhR in melanoma remains unclear.

We show here that melanoma cells functionally express AhR and that the AhR pathway activation is able to affect the cellular inflammatory response. We hypothesize that activation of cell intrinsic AhR signaling attenuates melanoma cell responses to inflammatory and DNA damage-induced stress, thus fostering their adaption to a changing microenvironment during disease progression and facilitating tumor cell survival and metastatic spread.

In accordance with our hypothesis we found that activation of AhR via formylindolo[3,2-b]carbazole (FICZ), a tryptophan photoproduct and endogenous AhR ligand, attenuates tumor necrosis factor-alpha (TNF-alpha)- and lipopolysaccharide (LPS)-induced immune responses in primary and transplantable HCmel 12 melanoma cells in vitro. Upon AhR activation, melanoma cells show reduced release of pro-inflammatory chemokines and a less invasive phenotype upon stimulation with TNF-alpha and LPS in a transwell migration set-up. Interestingly, we were also able to show that both LPS and TNF-alpha lead to an upregulation of AhR. Confirming our first results, this anti-inflammatory and dampening effect of AhR activation via FICZ was not visible in AhR−/− cells generated by CRISPR/Cas9 genome editing. Moreover we identified c-kit (CD117) as a target of ligand-dependent AhR signaling in melanoma, indicating an additional effect of AhR on cell proliferation and differentiation.

In vivo, we observed a less aggressive phenotype in AhR-deficient tumors concomitant with delayed growth kinetics and significantly decreased number of pulmonary metastases upon transplantation in immunocompetent C57BL/6 mice. AhR−/− tumors also showed a stronger pigmented phenotype in contrast to wild-type melanomas. Thus, our data suggest a critical role for AhR in the regulation of inflammatory responses in melanoma and phenotypic plasticity making it a promising target for novel therapeutic intervention in malignant melanoma.

P227 | HMGB1 in phenotypic plasticity: visualizing a putative resistance mechanism

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Introduction: In previous studies our group uncovered the mechanism of UV-induced metastatic spread of melanoma cells via DNA damage in keratinocytes, release of the High-mobility group box 1 (HMGB1) protein and its signaling through Toll-like receptor 4. We believe adverse environmental stimuli such as inflammation also promote the nuclear translocation and subsequent release of HMGB1 in melanoma cells themselves, providing an autocrine signaling loop. As a result, the cell undergoes a phenotypic switch, enabling it to adapt to a changing microenvironment, a feat essential for the ability to metastasize. In this project we aim to characterize this process of phenotypic change and uncover its cellular mechanism as a basis for novel treatments of melanoma and other neoplastic diseases.

Results: Creation of retroviral constructs for HMGB1-TagGFP and LC3B-TagRFP fusion proteins are performed with our ligation-independent-cloning system. Successful integration was confirmed via both Western Blot and fluorescence microscopy. Transgenic HCmel 12 cells expressing the HMGB1-TagGFP fusion protein show a strong translocation of HMGB1 into the cytosol upon treatment with TNF-alpha. Co-stimulation with the autophagy inductor Rapamycin leads to a marked increase of HMGB1 translocation. Transplantation experiments of the HMGB1 transgenic HCmel 12 cells in C57BL/6 mice reveal an almost complete translocation of HMGB1 into the cytosol.

Discussion: Our results indicate a pivotal role of HMGB1 as well as autophagy in the process of phenotypic plasticity. Corresponding to our previous findings, melanoma cells themselves also employ these evolutionary conserved countermeasures to stress. Further analysis will elucidate the role of HMGB1 shuttling and signaling in phenotypic plasticity as well as the impact of autophagy and other metabolic pathways thereon.

MISCELLANEOUS

P228 | Assessment of the compatibility of a non-adhering dressing and CNP foam during NPWT in vitro

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Introduction: NPWT has been shown to be clinically effective in the treatment of chronic-stagnating wounds. In vitro studies suggest that positive effects of NPWT result from the recruitment of cells to the wound site. It could be shown that the dressings used for NPWT exhibit different effects, cells especially show a significant tendency to grow into large-pored foams. We have used an in vitro model for NPWT to investigate the effects of the combination of non-adhering dressing and large-pored PU foam dressing on fibroblasts.

Methods: Non-adhering dressing samples (Lomatuell® Pro, Lohmann & Rauscher) and large-pored PU foam dressing (CNP® foam, Lohmann & Rauscher) were placed on fibroblast 3D-cultures. The assembly was positioned in a 6-well-plate and sealed with a vacuum-applicator-lid (VAL). VALs were connected to medium supply and vacuum pump. Experiments were carried out at −80 mm Hg for 48 hours. Histology

specimens were stained with haematoxylin/eosin and fibroblasts were detected using anti-vimentin-antibodies. Cell viability and ingrowths of cells into samples was determined.

Results: Fibroblasts responded to subatmospheric pressure by migrating in direction of the applied vacuum. Using the combination of non-adhering dressing and PU foam dressing samples during NPWT at −80 mm Hg did not affect fibroblast migration in vitro.

Conclusions: It could be shown that the combination of non-adhering dressing and PU foam dressing demonstrates good cell compatibility and does not negatively affect cell viability. Moreover, the combination of non-adhering dressing and PU foam dressing samples allowed induction of fibroblast migration in direction of the applied vacuum during NPWT at −80 mm Hg.

P229 | Comparison of the antibacterial effects on *Pseudomonas aeruginosa* and a *Staphylococcus aureus* biofilm of a class III PHMB releasing foam and a class IIb PHMB non-releasing foam

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Introduction: Chronic wounds are often colonized by different microorganisms, the most prominent being *Staphylococcus aureus* and *Pseudomonas aeruginosa*. PHMB-containing dressings have been shown to effectively inhibit bacterial progeny. However, bacteria do not act alone and the concept of biofilm formation and presence is now widely accepted. Therefore, current research targets antibiofilm strategies to restore an optimal wound-healing environment. A combined treatment approach involving debridement and the addition of antibacterial agents may then provide the highest success rates. Here, the efficacy of a new PHMB-releasing PU foam (class III product, MDD 93/42/EEC) against a *Staphylococcus aureus* biofilm was evaluated in vitro and compared to a non-releasing class IIb (MDD 93/42/EEC) PHMB foam (antimicrobial effect in the foam only). Moreover, antibacterial activity was evaluated in a direct contact method as well as by an extraction-based method against *S. aureus* and *P. aeruginosa*.

Methods: Antibacterial activity of the PHMB-releasing PU foam (Suprasorb P + PHMB; Lohmann & Rauscher) and a PHMB-non-releasing foam (DracoFoam Infekt, Dr. Ausbüttel & Co. GmbH) against *S. aureus* and *P. aeruginosa* was tested according to JISL1902:2008. In addition, extracts from the dressings were obtained (extraction ratio: 1 g:50 ml, extraction conditions: 24 hours at 37°C). Effect of the extracts on microbial growth was monitored by microplate laser nephelometry (MLN). *S. aureus* biofilm was cultivated on glass plates, covered directly with dressings or covered with dressings after treatment with Debrisoft (Lohmann & Rauscher), and incubated for 24 hours at 37°C. Biofilm was quantified directly after dressing removal and following 48 hours regrowth period using the alamar blue assay.

Results: The new class III PHMB-releasing PU foam displayed complete inhibition of both, *S. aureus* and *P. aeruginosa* while the non-releasing class IIb PHMB foam only exhibited a slight antibacterial effect. Additionally, the extract of the class III PHMB-releasing PU foam demonstrated a distinct inhibition of bacterial growth (IC50-*S. aureus*: 0.41% and IC50- *P. aeruginosa*: 14.8%). In contrast, no antimicrobial active amounts of PHMB were released from the class IIb PHMB foam. After previous treatment with the wound debrider Debrisoft, the new class III PHMB-releasing PU foam efficiently reduced the *S. aureus* biofilm and significantly less viable bacteria were observed. The class III PHMB-releasing PU foam exhibited a significantly higher reduction of biofilm compared to the class IIb PHMB foam after debridement.

Conclusions: It was found that the new class III PHMB-releasing PU foam exhibits a strong antibacterial activity against prominent microorganisms in chronic wounds. Moreover, it could be shown that the class III dressing is able to release its antimicrobial agent in active quantities and further to reduce biofilm after debridement with Debrisoft in-vitro. Hence, it can be expected to exert beneficial effects in stagnating wounds and promote healing with the combination of debridement and antiseptic treatment, here, Debrisoft and the class III PHMB-releasing foam.

P230 | New established human three-dimensional oral mucosa model for pharmacological studies in wound healing

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This newly engineered organotypic oral mucosa skin model is a viable tool to investigate biology and wound healing process of oral mucosa initiated by laser treatment and is qualified for pharmacological studies. It is suitable as an in vitro replacement method to animal experiments for drug targeting and testing new therapeutics or studying wound healing processes in oral mucosa.

The peculiarity of that skin model is the adaption of the culture conditions due to the experimental project such as testing pro-vitamin B5 containing ointments. For that purpose the generation of a modified culture media free of dexpanthenol and proliferation enhancing additives associated as a deficient culture media is required.

3D skin models were irradiated with the non-sequential ultrapulsed fractional carbon dioxide (CO2) laser (energy 100 mJ, 100 Hz, 1 pass). During laser irradiation, culture medium was removed. Radiation intensity was kept under equal conditions by fixing the laser head on a tripod. Lesional 3D skin equivalents were subsequently treated topically with 5% dexpanthenol containing verum, or placebo ointment without dexpanthenol. Wound healing was examined using histological analysis.

In conclusion, we demonstrated that CO_2 laser treatment is an efficient and rapid method for providing a model of epidermal wounding on human skin equivalents which serve as a standardized in vitro model for human wound healing. Topical treatment with 5% dexpanthenol containing ointment enhanced wound closure in oral mucosa equivalents treated with non-sequential fractional ultrapulsed CO_2 laser compared to placebo. This novel 3D wound healing skin model system is useful to find biomarkers for wound healing in skin as well as to investigate cellular events associated with abnormal wound healing (eg, chronic, non-healing wounds). It allows reliable data and can be applied for comparative studies analyzing the effect of topically or systemically applied compounds on wound healing hereby avoiding animal experiments or clinical trials in humans.

P231 | Epidermal differentiation, cytokines, IgE and filaggrin in atopic dermatitis (AD), classical prurigo nodularis (PN) and PN in AD

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AD and PN are different inflammatory skin diseases characterized by intense itching. PN may also occur as a manifestation of AD. Progress has been made in understanding AD, whereas the pathology of PN is largely unknown. The aim of this project is to compare these three groups with healthy controls in terms of clinic, histology, epidermal differentiation, immunology, and genetics for a better classification and therapy. Groups of 8-10 patients were clinically classified. Histology, epidermal differentiation markers, and cytokine expression were determined in skin biopsies. IgE and filaggrin mutations were determined in blood samples. Prurigo nodules in non-AD were mainly localized on the lower legs, whereas the nodules in AD were more spread over the entire legs. In AD the eczema lesions were localized in the classical areas inner knees and elbows. Epidermal thickness was increased in lesional AD, but much more in prurigo nodules in AD and especially in prurigo nodules in non-AD compared to control. In AD and in PN in AD, but not in PN in non-AD, spongiosis was noted. The number of inflammatory cells was increased in all three groups, and especially in PN in non-AD. Involucrin and loricrin expression were increased and filaggrin expression was reduced in all three groups. Interestingly, loricrin expression reached the highest levels in PN in AD and was significantly different from PN in non-AD and AD. Epidermal IL-4 and IL-13 expression were increased in all three groups, but reached significant levels compared to control only in the prurigo forms. IgE-levels were highly significant elevated in AD and borderline values were obtained in the prurigo forms. Three out of 10 patients in AD and one in 10 controls, but none of the patients in the prurigo forms, showed filaggrin mutations. These results show significant differences between the three diseases. Filaggrin mutations and increased IgE-levels were only seen in AD. Extreme epidermal thickness and severe inflammation were only noticed in the prurigo forms. PN in AD and PN in non-AD were distinguished by spongosis and the increase in loricrin expression.

P232 | Involvement of NF-κB c-Rel in systemic sclerosis

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Systemic sclerosis (SSc) is a rare idiopathic connective tissue disease with vascular, immune and fibrotic features. Various cytokines and growth factors provoke the characteristic uncontrolled fibrotic response leading to fibroblast proliferation and myofibroblast conversion associated with excessive extracellular matrix (ECM) protein production. Limited therapeutic options and high mortality highlight the need for a better understanding of SSc pathogenesis. Aberrant NF-κB expression and activity have been implicated in this process. Recently, the c-Rel subunit of NF-κB has been shown to influence epidermal homeostasis, fibrotic processes and autoimmune diseases in mice.

Following TGF-β1 stimulation as fibrotic trigger, cultured dermal fibroblasts showed myofibroblast differentiation (including α-smooth muscle actin and plasminogen activator inhibitor-1 expression). Concomitantly, analysis of subcellular compartments revealed increased nuclear c-Rel levels after TGF-β stimulation compared to unstimulated dermal fibroblasts suggesting increased c Rel activity after TGF-β stimulation. Efficient c-Rel knockdown of about 60% by transient siRNA transfection did not impair cell viability after TGF-β stimulation. Thus, while further analyses regarding phenotypical and functional roles will be necessary, our results thus far are consistent with a role of c-Rel in systemic sclerosis (patho)physiology.

P233 | Stratum corneum lipid profiling in atopic dermatitis with respect to filaggrin mutations

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Poor epidermal barrier function due to inherited factors such as filaggrin (FLG) deficiency and/or environmental exposures are a major pathophysiological peculiarity in atopic dermatitis (AD). Despite much research, the multifaceted roles of lipids for barrier function and AD pathogenesis and the impact of FLG mutations on skin lipid composition are incompletely understood. So far no systematic study on stratum corneum (SC) lipids has been carried out.

We systematically characterized the SC lipid profiles (123 ceramides, 61 dihydroceramides, 82 6-hydroxyceramides, 72 phytoceramides and 12 free fatty acids) and skin physiology parameters at three body sites in a collection of 10 AD patients and 10 controls matched for

age, sex and FLG mutation status (FLGmut/ FLGwt) using SFC-MS/MS (Supercritical Fluid Chromatography coupled with MS/MS in sMRM mode).

FLGmut individuals with and without AD had significantly higher skin pH values than individuals not carrying FLG mutations. Multidimensional scaling revealed considerable differences in the lipid profiles of the forehead and the proximal forearm.

On a false discovery rate (FDR) adjusted significance level, 8 lipids showed significantly lower lipid levels in AD patients at the cubital fossa and the proximal lower arm. (6 phytoceramides, 2 6-hydroxyceramides).

There were no significant differences in lipid levels between FLG mutation carriers and non-carriers.

AD patients show markedly lower levels of several ceramides both at dry and moist skin sites, however, there appears to be no clear relationship with FLG mutations status. There appears to be no quantitative differences with regard to other lipid classes.

P234 | Long lasting effect of skin microbiome modulation induced by probiotic solution application

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The human body is host of a rich microbial community. The skin is colonized by a large number of microorganisms, most of them are beneficial or harmless. However in many chronic skin diseases the composition of the skin microbiome differs from that of healthy skin. Gut microbiome modulation, through fecal transplantation, have proven as a valid therapeutic strategy in diseases such as *Clostridium difficile* infections. Therefore techniques for the directed modulation of the human skin microbiome may become a potential therapeutic strategy for the treatment and study of chronic skin diseases which are associated with a dysbiosis of the skin microbiome.

We have demonstrated that we can modulate the skin microbiome composition. We show that after sequential applications of a donor skin microbiome, the composition of the recipient skin microbiome becomes similar to the donor. We followed 12 subjects for multiple weeks. After interrupting the application of a donor microbiome, we observe an initial phase dominated with abundance of donor strains, and we observe a large scale microbiome re-organization that lasts up to several weeks.

Directly modulating the skin microbiome by applying natural skin bacteria is possible. The observed effect is longer lasting than the application of the bacteria. This opens opportunities to develop microbiome-based therapies for diseases associated with strong alterations of the skin microbiome.

P235 | Skin surface stripping and DNA extraction for high throughput NGS analysis of the acne vulgaris-associated microbiome

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Recent insights into the biology of the human microbiome hold great potential for the development new therapeutic strategies. To determine the efficacy of such strategies, correct and reliable information about the composition of the microbiome must be obtained. Advances in NGS technologies provide researchers with cost effective methods for detailed microbiome analyses. However, the high sensitivity of this method requires careful study planning and experimental design (Meisel et al., 2016). Among other factors, different methods for sample acquisition should be taken into account depending on the target tissue, the type of disease and consequently the different bacterial populations to be studied. An attractive model disease in dermatologic microbiome research is acne vulgaris. Recently, many studies reported a connection between the clinical severity of acne and the population of *Propionibacterium acnes*. The *P. acnes* community predominantly colonizes the follicular canal of the skin sebaceous units. Therefore, an adequate sampling strategy is required to analyze *P. acnes* populations.

Cyanoacrylate stripping is a method that can efficiently sample the content of the follicle. Sampling individual microcomedones from a strip and has been the most frequently applied method to study the *P. acnes* population. However, this method is not suitable to study larger sample numbers. We therefore hypothesized that it is possible to sample the *P. acnes* population in a high throughput manner using a skin stripping method.

Different ways of extracting DNA from the strips and the compatibility with subsequent PCR amplification and NGS analysis were investigated. The main challenges were PCR inhibition and low DNA concentration. This problem was addressed using a bead-based DNA purification method which is compatible with common automation systems.

We then compared our new method of sampling the microbiome of skin follicles with swab sampling, an established method for sampling the upper layers of the skin and easily accessible for use in high throughput NGS.

We demonstrate that the communities sampled by strips and swabs are different, confirming that the collection method influences greatly the screened populations and the overall result. Cyanoacrylate strip method proves better for sampling *P. acnes* populations presented in the follicular canal while skin swabs are more suitable to sample the skin surface microbiome.

Literature: Meisel, J.S., Hannigan, G.D., Tyldsley, A.S., SanMiguel, A.J., Hodkinson, B.P., Zheng, Q., and Grice, E.A. (2016). Skin microbiome surveys are strongly influenced by experimental design. J. Invest. Dermatol. 136, 947-956.

P236 | Cellular mechanism of action of IgE-specific immunoadsorption in treatment of patients with severe atopic eczema

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IgE-specific immunoadsorption (IA) is an effective treatment for severe forms of atopic eczema (AE) in some patients. However, mechanisms behind this therapeutic approach and biomarkers predicting the therapeutic outcome are not yet known. This study is aimed at detecting the impact of IA on cellular level and clinical outcome. Ten patients with severe AD (SCORAD>40) and highly elevated IgE levels (IgE>2000 IU/mL) were included in the study. Every patient received a total of 10 IgE-specific IA sessions that were conducted in three intervals with a break of 2 weeks between each interval. A follow-up examination was performed 4 weeks after the last session. Frequencies of different immune cell populations and their Fc receptor expression profiles were monitored by flow cytometry at the beginning and the end of the first interval to detect short-term effects and at the end of the last interval, respectively, to evaluate mid-term effects. Finally, the follow-up analysis monitored potential long-term effects.

As expected, IA decreased IgE levels, which tended to increase again overnight and showed no differences to the level prior to IA at follow-up. Despite this marginal short-term effect on serum IgE levels, IA was clinically effective with an average reduction of the SCORAD of 35.74% at the follow-up. Even though the number of FcRIa-positive basophil granulocytes remained unchanged, FcRIa receptor density decreased. Moreover, not only the expression of CD23 on B cells but also the receptor density decreased. This effect could be mainly attributed to memory and transitional B cells, respectively.

In conclusion, although reduction of IgE levels by IgE-specific IA is not persistent, the improvement of SCORAD and patient well-being is long-lasting in some cases. Even though the underlying mechanisms are not yet fully elucidated, effects of IA at the cellular level might contribute to the clinical efficiency.

P237 | Unexpected cutaneous benefits of pleasant smell: olfactory receptor stimulation promotes human hair growth

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Chemical signals acting on specific receptors such as olfactory receptors (OR) are the most common and precise form of communication. Many non-neuronal cells, incl. keratinocytes, engage in chemosensation via OR stimulation to regulate physiological cell functions, eg, OR2AT4-keratinocyte proliferation and migration in vitro. Therefore, we asked whether OR2AT4 also plays a role in human hair follicle (HF) biology. By immunofluorescence microscopy and qRT-PCR, we found that human anagen VI scalp HFs transcribe the OR2AT4 gene and most prominently express OR2AT4 protein in suprabulbar outer root sheath (ORS) keratinocytes.

We investigated next, the effect of a specific OR2AT4 agonist, the synthetic sandalwood oil, Sandalore®, on human HF cycling ex vivo. This showed that, compared to vehicle-treated HFs, specific OR2AT4-stimulation by Sandalore® significantly retarded spontaneous catagen development. This catagen-inhibitory effect was mirrored by the fact that OR2AT4 stimulation with this odorant significantly decreased the number of apoptotic (TUNEL+) and caspase-3+ hair matrix keratinocytes. All these effects of OR2AT4 stimulation were partially counteracted by the co-administration of a specific antagonist, Phenirat®. OR2AT4-dependency of these effects was documented by showing that, despite 6 days of agonist treatment (Sandalore®), catagen was induced prematurely in OR2AT4-silenced human anagen HFs, compared to scrambled oligo-treated HFs, as assessed by quantitative hair cycle histomorphometry.

To further dissect the mechanism by which Sandalore® affects HF growth, we examined the expression of TGFβ2 (potent catagen inducer) and IGF-1 (anagen-maintaining growth factor) after 6 days of treatment. This showed a significant decrease in the expression of TGFβ2 (=catagen inducer) and a significant increase of mRNA and protein expression of anagen-promoting and apoptosis-suppressing IGF-1 in Sandalore-treated HFs. Selective OR2AT4 silencing enhanced hair matrix keratinocyte apoptosis (TUNEL+ and caspase-3+) and decreased IGF-1 expression while TGFβ2 expression was unchanged, suggesting that OR2AT4 activation primarily impacts on human HF cycling via up-regulating IGF-1.

The anti-apoptotic effect of Sandalore® on hair matrix keratinocytes was confirmed by microarray analysis, since pro-apoptotic genes were significantly downregulated (eg, protein prune homolog 2) and anti-apoptotic genes significantly upregulated (ie, proline-rich AKT1 substrate 1) under Sandalore® stimulation compared to vehicle-treated control HFs. We confirmed these data on the influence of Sandalore stimulation by phosphokinase assay, in particular for the IGF-1 pathways where different kinases were more phosphorylated [proline-rich AKT1 substrate 40 (PRAS40)] compared to the vehicle In summary, we show for the first time that human HFs can "smell", ie, engage in OR-mediated chemosensation: OR2AT4 not only is differentially expressed in human HF epithelium and functions to prolong anagen ex vivo, but continuous OR2AT4 signaling by (as yet unknown) endogenous ligands is also essential for anagen maintenance. Moreover, Sandalore® may be recruited for the adjuvant management of HF cycling disorders, namely those associated with telogen effluvium.

P238 | Fluoxetine promotes hair follicle pigmentation: a new anti-greying strategy?

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Besides regulating complex central nervous system functions, the neurotransmitter, serotonin (5-HT), acts in the periphery by modulating receptors involved in pain, cutaneous vasodilatation, inflammation, and pruritus. 5-HT is produced and metabolized by different cell types, including melanocytes, which also express cognate receptors (5-HT1-4R). 5-HT itself reportedly promotes pigmentation in vitro (human melanocyte cell lines), and in vivo (mice), and selective serotonin reuptake inhibitors (SSRI)fluoxetine, can stimulate melanin production in murine HFs. Therefore, we asked whether fluoxetine exerts any effect on human HF pigmentation.

To address this question, we microdissected human pigmented, full length scalp HFs in anagen VI from two female facelift surgery patients and treated them with fluoxetine (1 μmol/L and 100 nmol/L) for 48 hours in serum-free organ-culture. Quantitative (immuno-) histomorphometry for standard HF pigmentation parameters revealed that fluoxetine increased melanin production in the hair matrix (Masson-Fontana histochemistry) and protein expression of the key melanogenesis-stimulatory neurohormone, alpha-melanocyte-stimulating hormone (a-MSH), in the HF outer root sheath (ORS) ex vivo. Instead, in fluoxetine-treated human skin organ cultures (derived from two additional female patients), epidermal melanocytes appeared to be unaffected by this SSRI.

Next, we asked whether fluoxetine (1 μmol/L, 100 nmol/L) can even stimulate some degree of re-pigmentation of white HFs over 6 days of HF organ culture. Quantitative (immuno-)histomorphometric analyses for corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), pre-melanosome protein (gp100), alpha-MSH, melanin production (by Masson-Fontana) and tyrosinase activity in situ were run. Although, as expected, most of these pigmentation-related readout parameters were not significantly affected by fluoxetine treatment, white anagen VI HFs revealed upregulated immunoreactivity for CRH in the ORS, and for gp100 in the matrix of white HFs. Most importantly, actual melanin production in the HF pigmentary unit was also significantly induced in white anagen VI HFs, as assessed by Masson-Fontana histochemistry.

Taken together, these data suggest a new role for fluoxetine in human skin physiology and pathology: Fluoxetine may not only retard HF depigmentation by stimulating the HF pigmentary unit, but also can promote the re-pigmentation of white HFs. Our data also confirm that "white" human scalp HFs still retain considerable residual re-pigmentation potential, which can be targeted therapeutically. Therefore, fluoxetine and other SSRIs deserve to be fully explored as candidate anti-hair greying agents.

P239 | Investigation into ammonia molecules diffusing from the skin surface and their relation to lactate levels and pH of the skin surface

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For in vivo investigation of the skin molecules that can diffuse from the skin surface into the environment at room temperature may be very useful. Among such molecules is ammonia that may not only be an interesting parameter for the biophysical investigation of the skin, but also a parameter for different ammonia sources in the skin such as sweat production or skin barrier function. In order to apply ammonia as parameter for investigation of the skin it is required to gain knowledge about its relation to the molecular environment in the skin and in particular within the skin surface. In the present investigation we assess ammonia diffusing freely from the skin surface and investigate its relation to lactate anions from the skin surface as well as to the pH of the skin surface.

Overall 43 volunteers (20 men, 23 women) were included into the study after obtaining informed written consent. All investigations were performed in summer time under standardized room conditions. The forearm was the site of investigation. For ammonia determination the molecules were first trapped from the air above the skin surface using gradient grade water while assuring that there was no direct contact between the water and the skin surface. Afterwards the ammonia molecules were quantified using a photometric assay. Lactate was determined by rinsing the skin surface using gradient grade water and by consecutive quantification using again a photometric assay. The pH of the skin surface was assessed by means of a standard glass electrode. For statistical evaluation median parameters were calculated. Also, after assessment of the distribution of the data statistical comparisons between the values assessed in men and women and a correlation analysis to assess the interrelationship between the parameters were performed.

The results revealed median ammonia levels of 1.85 ng/cm^2 min diffusing across the skin surface. Comparison between men and women showed significant differences (P<.001). The median mass of lactate rinsed from the skin surface was 1.73 μg/cm^2 also showing significant differences between men and woman (P=.004). The median pH values were 4.84 showing significant differences between men and women (P=.016), too. The analysis of correlation revealed a significant positive correlation between ammonia molecules and lactate (r=.464; P=.002). There was a significant inverse correlation between ammonia and skin surface pH (r=−.392; P=.009) as well as between skin surface pH and lactate (r=−.378; P=.014).

The results obtained indicate that the diffusion of ammonia from the skin surface into the environment is related to the lactate anions as well as the pH of the skin surface. The positive correlation between ammonia and lactate may indicate that ammonia trapped over the skin surface is due to ammonium lactate within the skin surface. The inverse relation between lactate and skin surface pH might indicate that

lactate has a direct pH decreasing effect on the skin surface. Given that the investigation was performed in summer, the significant higher ammonia and lactate values accompanied with significant lower pH values in men when compared to women suggest that sweat might have been the main source of the molecules assessed. Further studies in winter and studies assessing the intracellular amount of lactate anions in corneocytes may help to discriminate the influences of sweat and stratum corneum on ammonia diffusion across the skin surface.

P240 | A novel treatment principle in anti-hirsutism management: an osteopontin-derived peptide potently inhibits human hair growth in vitro and in vivo

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Undesired hair growth (hirsutism, hypertrichosis) can cause major psychological distress. Since only few, and then often unsatisfactory therapeutic options are currently available, new treatment strategies need to be developed. Given that the multifunctional, immunomodulatory glycoprotein, osteopontin, reportedly is expressed by rat hair follicles (HFs) only during catagen, we hypothesized that osteopontin-derived peptides may inhibit human hair growth and have tested this hypothesis, using a newly generated, short modified osteopontin-derived peptide (FOL-005). In microdissected, organ-cultured human scalp HFs, FOL-005 highly reproducibly inhibited hair shaft production and induced premature HF regression (catagen). This was confirmed in organ-cultured, full-thickness human scalp skin from 6-9 subjects, where FOL-005 (15 nmol/L, 150 nmol/L) significantly promoted catagen development, along with increased hair matrix keratinocyte apoptosis. When human male scalp skin was transplanted onto SCID/beige mice (three 3 mm^2 grafts per mouse) and FOL-005 was injected intracutaneously, this significantly decreased the number of hairs growing per graft and prematurely induced catagen in vivo compared to vehicle-treated control xenotransplants. Moreover, FOL-005 administration potently counteracted the hair growth-promoting effects of minoxidil, one of the strongest hypertrichosis-inducing agents. There was no morphological evidence of FOL-005-induced HF-toxicity, and a standard battery of toxicological tests revealed no overall FOL-005 toxicity. Microarray analysis revealed decreased transcription of FGF7, a known hair growth-promoter (confirmed by qRT-PCR and immunohistochemistry). Co-treatment of FOL-005 (15 nmol/L) with recombinant FGF7 (100 ng/mL) in microdissected, organ-cultured human scalp HFs abrogated the catagen promoting effects of FOL-005, consolidating FGF7 as a bonafide target of FOL-005. These data identify this osteopontin-derived peptide as a potent, novel inhibitor of human hair growth in vitro and in vivo, which deserves clinical testing as a new treatment principle for excessive hair growth (hirsutism, hypertrichosis).

P241 | Desmoglein-specific immunoadsorption abolishes the pathogenic effect of serum IgG from patients with pemphigus vulgaris

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Pemphigus vulgaris (PV) is a life-threatening autoimmune blistering skin disease affecting skin and mucous membranes. Patients predominantly develop autoantibodies against desmoglein 3 and 1 (Dsg 3, Dsg 1) that reduce cell-cell adhesion between keratinocytes. Treatment of PV is based on long-term systemic immunosuppression. Recently, the B cell-depleting anti-CD20 monoclonal antibody rituximab has been shown to induce remission in about 80% of PV patients. In severe PV, removal of serum autoantibodies appears to be a rational approach in the initial treatment phase. Adjuvant immunoadsorption has previously been shown to be effective in treatment of PV patients. Its use has been limited, however, by the concomitant removal of protective antibodies. Subsequently, we developed prototypic immunoadsorbers containing recombinant Dsg 3/Dsg 1 ectodomains. Here, we show that these adsorbers remove the pathogenicity of the PV IgG fractions against the entire Dsg 3 (and Dsg 1) ectodomain in vitro and in vivo using three different pemphigus models: a desmosome degradation assay, a keratinocyte dissociation assay, and a neonatal mouse model. Dsg 3- and Dsg 1-depleted IgG of three PV patients did not induce internalization of Dsg 3, did not cause acantholysis in the dispase-based dissociation assay, and did not lead to macroscopic or microscopic blister formation when injected into neonatal mice (n=3), in contrast to the Dsg 3-specific IgG fractions, eluted from the column, which showed a concentration-dependent internalization of Dsg 3, an increased fragmentation of keratinocyte monolayers, and induced macroscopic blistering in neonatal mice. In summary, immunoadsorption using recombinant Dsg 3 and Dsg 1 ectodomains was shown to completely abolish the pathogenic effect of PV sera both in vitro and in vivo. These data also demonstrate that anti-Dsg 3 antibodies alone are sufficient to explain major immunopathological and clinical characteristics of the human disease in vitro and in vivo.

P242 | P-cadherin-mediated signaling: a novel, clinically relevant differential modulator of matrix metalloproteinase activity in human skin physiology?

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P-cadherin, a cell adhesion molecule previously recognized in skin biology primarily for its role in murine hair follicle (HF) development, was recently found to also exert major roles in the control of human hair growth through signaling via the canonical WNT/β-catenin pathway and to be a novel regulator of melanocyte activity and melanogenesis. Loss-of-function mutations in CDH3 gene, which encodes P-cadherin, result in two autosomal recessive disorders associated with hypotrichosis and excessive percentage of HFs in catagen or telogen. In order to delineate additional pathways by which P-cadherin may exert its complex roles in human hair biology, we performed microarray analysis of P-cadherin-silenced human HFs compared to control HFs treated with scrambled oligonucleotides. These microarray results revealed that matrix metalloproteinases (MMPs), ie, extracellular matrix-degrading proteases, are differentially modulated by CDH3 silencing: MMP-1 and -3 transcriptions are up-regulated, while MMP-9 transcription is down-regulate. This differential regulation of intrafollicular MMPs transcription by P-cadherin was independently confirmed by qRT-PCR in two different labs in mRNA extracts from 6 different individuals. Moreover, qRT-PCR analysis of P-cadherin silenced HFs also suggested differentiated expression of tissue inhibitors of metalloproteinases (TIMP-1, -2, -3, -4), ie, key regulators of MMP activities. Therefore, we hypothesized that P-cadherin is an important, novel regulator of MMP activity in normal human tissue physiology and that P-cadherin-controlled differential changes in MMP activity may impact on human HF cycling (previously, we had shown that HF cycling in mice is associated with significant changes in HF-related MMP activities). Our preliminary data show that P-cadherin silencing of organ cultured human scalp skin also increased MMP-1 protein expression in the dermis and in precortical hair matrix keratinocytes ex vivo. We shall report additional results on the impact of P-cadherin knockdown on MMP expression and activity in human skin and HFs, combining immunohistochemistry with in situ zymography, complemented with MMP promoter activity assays. If confirmed by these additional results, our study is expected to reveal a novel, clinically relevant, topobiological control of MMP biology in human skin, the "P-cadherin-MMPs connection".

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