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Allergy

P001

Characterization of T cells specific to the human autoantigen Hom s 2 in atopic dermatitis on single cell level

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Autoreactivity to several human proteins has been described in a subgroup of patients with atopic dermatitis (AD) and may contribute to the pathomechanism. First described on IgE level, we were recently able to show autoreactivity against human proteins by T helper and also cytotoxic T cells *in vitro*. More precisely, we generated and characterized T helper as well as cytotoxic T cell clones specific for Hom s 2, the alpha-chain of the nascent polypeptide-associated complex (α-NAC). Now, we identified major T cell epitopes of Hom s 2 presented via MHC class I and MHC class II, allowing the production of MHC tetramers and thereby enabling the detection of specific T cells.

Here, we used the cell analysis technique Chipcytometry (www.chipcytometry.com) to perform deep characterization of tetramer-binding T cells on single cell level. By subsequent fluorescence staining/bleaching steps, immobilized cells are analyzed by a comprehensive marker set, providing information about T cell subset and displaying the tetrameric staining pattern in detail.

By this means, we discovered terminally differentiated autoantigen-specific T cells (Tetramer+/CD45R0+/CD27−) in sensitized patients with atopic dermatitis.

P002 (O30)

Serum levels of IL-31 are increased in mastocytosis and correlate with disease severity in adult patients

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Mastocytosis is characterized by clonal expansion of mast cells (MC) in tissues, particularly in skin and bone marrow. The expansion of MC is variable resulting in a broad spectrum of different disease categories, which encompass benign subtypes like cutaneous mastocytosis and advanced categories like MC leukemia. Several biomarkers are known to correlate with MC burden in mastocytosis. The best-characterized marker is the MC-derived enzyme tryptase, which is also used in clinical routine to monitor patients with mastocytosis. In recent years, it has become evident that the novel cytokine IL-31 plays an important role in the induction of chronic skin inflammation. In the present study, we aimed to clarify whether IL-31 is increased in mastocytosis and whether IL-31 levels differ between disease categories.

Expression of IL-31 was analyzed in serum of 50 patients (38 adult and 12 pediatric patients) with different categories of mastocytosis. Serum levels of IL-31 were significantly increased in patients with mastocytosis compared to healthy controls. Interestingly, within the group of adult patients, those with advanced categories exhibited significantly elevated IL-31 levels compared to those with non-advanced categories. Furthermore, IL-31 correlated with tryptase levels as well as the percentage of MC infiltrates in bone marrow, and patients with MC infiltrates >15% showed significantly increased IL-31 levels compared to patients with infiltrates $\leq$15%. Surprisingly, we observed significantly increased IL-31 levels in pediatric patients with cutaneous mastocytosis compared to adult patients with cutaneous or indolent systemic mastocytosis.

To determine the cellular source of IL-31, we next analyzed skin and bone marrow biopsies of patients with mastocytosis by immunohistochemistry using specific antibodies against tryptase and IL-31. In both tissues, expression of IL-31 clearly colocalized with tryptase-positive MC, demonstrating that MC represent a major source of IL-31 in mastocytosis.

In conclusion, our results show for the first time that patients with mastocytosis express increased serum levels of IL-31. In adults, IL-31 correlates with severity of disease categories, tryptase levels and percentage of bone marrow infiltration. Our data provide evidence for involvement of IL-31 in the pathogenesis of mastocytosis and suggest exploiting IL-31 levels as potential diagnostic marker in adult patients.

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P003

Cellular sources of interleukin-10 during the resolution phase in contact hypersensitivity

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Contact hypersensitivity (CHS) is the classical murine model of allergic contact dermatitis. While the process of sensitization and elicitation is well investigated, mechanisms that determine the resolution of the inflammatory response are less well understood. Recent studies suggested a regulatory role of mast-cells (MC) and particulary MC-derived IL-10 (Grimbaldeston et al 2007) in the control of CHS, while others reported MC to be required for optimal CHS responses (Dudeck et al 2011). Based on these controversies we analysed the cellular sources of IL-10 during the resolution phase of CHS in the DNFB model using IL-10 transcriptional reporter mice (Vert-X). Neither at base line nor after allergen challenge MCs of the affected ear skin or the draining lymph nodes displayed IL-10 expression.

In contrast, a clear IL-10 signal was observed in the T cell compartment. IL-10 expression was observed in CD4+ T cells, especially CD25+, with a maximum during the resolution phase (i.e. 120 h after the challenge). In addition, a marked increase of IL-10 positive CD8+ cells was detected in the ear skin and to a lesser degree in the regional LN. The functional role of CD4+ T cells in regulating the CHS response was confirmed using MHC class II−/− mice that lack CD4+ T cells and displayed an augmented CHS response. Similarly, selective depletion of Treg (DEREG mice) prior to challenge resulted in an augmented CHS response, suggesting a dominant regulatory role of CD4+ T cells in attenuating the immune response to DNFB in CHS. Finally, the inhibitory role of T cell derived IL-10 was confirmed in mice with a T cell specific IL-10 deficiency (IL-10fl/fl CD4-Cre+). Corresponding to previous reports (Roers et al. 2004) these mice displayed an enhanced earswelling response and a delayed resolution of the CHS response as compared to Cre- controls.

In conclusion, our results do not support the assumption that mast cell derived IL-10 is involved in limiting the CHS response. Instead they confirm a crucial role of CD4+ T cells and suggest that also IL-10 producing CD8+ T cells may play an additional regulatory role in the resolution of CHS.

P004

Induction of IL-1β production in immature human dendritic cells depends on the alum preparation applied *in vitro*

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Allergen-specific immunotherapy (SIT) is a clinically effective therapy for IgE mediated allergic diseases. Adsorbance of allergens to aluminium hydroxide (alum) is used for many vaccines as adjuvant, including allergen specific immunotherapy. As adjuvant alum has been shown to induce IL-β production via the NALP3 inflammasome. The aim of this study was to analyze the effects of alum on immune cells of allergic donors *in vitro*. Therefore, immature monocyte-derived dendritic cells (DC) from such donors were pulsed with Phleum pratense or Betula verrucosa allergens or LPS in combination with or without alum, and were matured with pro-inflammatory cytokines or were left immature. The mature and immature DC were co-cultured with autologous CD4+ T cells to induce proliferative responses. Additionally, we incubated monocytes or immature DC with allergen or LPS in combination with or without alum from two different manufacturers and quantified the production of IL-1β by ELISA and quantitative Real-Time PCR. While treatment of mature allergen-pulsed or unpulsed DC with alum had no effect on their T cell stimulatory capacity, allergen+alum-pulsed immature DC induced a slightly enhanced proliferation of autologous CD4+ T cells *in vitro* compared to allergen-pulsed immature DC alone. Monocytes released similar amounts of IL-1β into the supernatants when they were stimulated with LPS alone or in combination with alum *in vitro*. Allergen or alum alone did not lead to IL-1β production in monocytes. In immature DC IL-1β mRNA expression was only slightly induced after treatment with LPS and alum compared to LPS alone. Using another alum preparation IL-1β production was readily induced by alum in allergen-treated DC. These data demonstrate that the effects of alum critically depend on the alum preparation used for analysis.

P005 (O15)

Low zone tolerance protects from Th1/Th17-mediated colitis

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Crohn's disease, one of the most common chronic inflammatory bowel diseases, is characterized by an inflammation, which affects the entire gastrointestinal tract and each intestinal layer. The mouse model of TNBS-induced colitis is a Th1/Th17-mediated immune response and pathophysiologically similar to Crohn's disease in humans. Previously, we demonstrated that epicutaneous and oral applications of subimmunogenic amounts of allergens (e.g. TNCB) results in low zone tolerance (LZT), which prevents the development of a contact hypersensitivity reaction (CHS), a CD8+ Tc1-mediated skin inflammation. In the current study, we addressed the question whether low amounts of allergens also affect Th1/Th17-dependent reaction and influence immune responses in the entire organism (e.g. gut) regardless of their route of application. Therefore, we have analyzed the effect of oral and epicutaneous treatments with low doses of allergens on the outcome of the TNBS-colitis und the underlying immune mechanisms. Notably, we found that repeated applications of oral as well as epicutaneous subimmunogenic doses of the hapten TNCB affected the course of the TNBS-induced colitis and significantly reduced the inflammation in the gut. These results were evaluated by use of mini-endoscopy (*in vivo*) to assess a panel of inflammatory parameters (vascularity, granularity, translucency of the gut wall, fibrin and consistency of stool) and histology (decreased infiltration of inflammatory cells, vessel density, and colon wall thickness, loss of goblet cells). In addition, a diminished hapten-specific T cell-proliferation and reduced Th1/Th17-cytokine production (IFN-γ, IL-2, IL-17) was observed after both, epicutaneous and oral tolerization, indicating an inhibition of the Th1/Th17-mediated colitis by LZT. Furthermore, we put into question the role and function of CD4+CD25+ regulatory T cells (Tregs) in LZT modulation of TNBS-induced colitis. Here, mice were treated with anti-CD25 mAb for Treg depletion prior to repeated epicutaneous or oral applications of low doses of contact allergens and subsequent colitis induction. In the absence of CD4+CD25+ Tregs, LZT failed to develop but rather a pronounced colitis response was observed. These results were determined by a significantly increased colitis score and a strong hapten-specific T cell response (vigorous T cell proliferation, Th1/Th17-cytokine pattern) as compared to control tolerized colitis mice, indicating a pivotal role for Tregs in colitis prevention by LZT. In summary, this study demonstrated that independent of the site of tolerance induction LZT to haptens results in an inhibition of CD8+ Tc1-mediated skin inflammation (CHS) as well as of CD4+ Th1/Th17-mediated colitis and that CD4+CD25+ Tregs are critically involved in the course of epicutaneous and oral LZT.

P006

Topical application of Δ9-tetrahydrocannabinol attenuates contact allergic inflammation through cannabinoid receptor 1/2-independent effects on keratinocytes

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Δ9-Tetrahydrocannabinol (THC), the active constituent of Cannabis sativa, exerts its biological effects in large part through the G-protein coupled CB1 and CB2 receptors expressed primarily on neurons in the brain and immune cells in peripheral lymphoid organs, respectively. However, THC also has CB1/2 receptor-independent effects. Because of its immune-inhibitory potential THC and chemically related cannabinoids are being considered for the treatment of inflammatory diseases including allergic dermatitis. Here we investigated the role of CB1 and CB2 receptors which are also expressed by epidermal keratinocytes for the anti-inflammatory activity of THC in an experimental model for allergic contact dermatitis. We show that topical application of THC effectively decreased the magnitude of contact allergic ear swelling and immune cell infiltration not only in wild type but also in CB1/2 receptor-deficient mice. As a likely mechanistic explanation we found that THC was able to inhibit the production of proinflammatory chemokines by epidermal keratinocytes derived from both wild type and CB1/2 receptor-deficient mice. Our results suggest that topical THC attenuates contact allergic inflammation at least in part by limiting the ability of keratinocytes to secrete immune cell recruiting chemokines in a CB1/2 receptor-independent manner. These insights have important implications for the future development of strategies to harness cannabinoids for the treatment of inflammatory skin diseases.

P007

Interleukin-33 promotes proliferation of mouse mast cells

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

P008

Cytokine responses induced by the human autoallergen thioredoxin

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Introduction: A subgroup of patients with atopic dermatitis (AD) shows IgE reactivity towards autoantigens, termed autoallergy. For one of these autoallergens, thioredoxin, cross-reactivity to its *Malassezia sympodialis* (*M. sympodialis*) homologue, Mala s 13, has been shown. *M. sympodialis* is a skin-colonizing yeast and about 50% of AD patients show specific IgE towards *M. sympodialis* extract. From *Malassezia*-sensitized patients thioredoxin-specific T cell clones have been generated and characterized recently. This study aimed to further elucidate the effects of thioredoxin on PBMCs, T cells and monocytes.

Methods: Cytokine induction in PBMCs, isolated CD4+ T cells and monocytes was analyzed by RT-qPCR and ELISA. Western Blot analysis was used to characterize the involved signalling molecules. T cell lines (TCLs) were generated from blood of *Malassezia*-sensitized AD patients to study the responsiveness towards MHC class II peptides from the thioredoxin protein sequence.

Results: We found induction of proinflammatory cytokines in PBMCs from sensitized patients as well as from healthy donors. The activation of these cells was accompanied by phosphorylation of the MAP-kinase p44/42, and the transcription factors NF-κB and STAT-3. TCLs, generated in the presence of thioredoxin, Mala s 13 or *Malassezia* extract, showed distinct cytokine profiles. Thioredoxin TCLs also showed proliferation when restimulated with selected thioredoxin MHC class II peptides.

Conclusion: Similar to other autoallergens, thioredoxin has the ability to cause secretion of proinflammatory cytokines in PBMCs from sensitized AD patients and healthy donors. The identified thioredoxin MHC class II epitopes will be further used to characterize the T cell response to this autoallergen.

P009

Induction of TSLP in murine skin does not require endogenous TNFα

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Thymic stromal lymphopoietin (TSLP) is a critical effector of allergic inflammation. It is unknown whether endogenous TNF-alpha (TNFα) is essential for TSLP production *in vivo*.

In the present study we analysed which mediators induce TSLP in murine KCs *in vitro*, whether acute skin irritation increases TSLP production *in vivo* and whether endogenous TNFα acts as an intermediary of TSLP production in these settings.

KCs from Bla6-wildtype and TNF-knockout(TNF-Ko) mice were stimulated for 24 h with reported enhancers of TSLP production. To assess the regulation of TSLP *in vivo*, the skin of Bla6-wildtype and TNF-Ko mice was subjected to acute irritation. TSLP quantification was performed by ELISA and by RT-qPCR.

TNFα and PMA were the most effective inducers of TSLP followed by IL-1β, while IL-33, IL-25, TLR3 ligand and calcitriol showed little or no effect. TSLP production was comparable for TNF-Ko and WT-KCs under most conditions. *In vivo* acute skin irritation by croton oil and SDS induced TSLP. Substantial amounts of TSLP were detected in serum 18 h post-irritation. No differences in TSLP levels were noted between TNF-Ko and WT mice.

Pro-inflammatory cytokines are the most potent inducers of TSLP in mouse KCs. Despite the potency of exogenous TNF to induce TSLP, endogenous TNF is neither indispensable for TSLP production nor seems an intermediary in settings of acute skin irritation.

P010

Effect of venom immunotherapy on expression of TIM-molecules on T cells and dendritic cells

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Venom immunotherapy (VIT) is a well established treatment that offers long-term protection from IgE-mediated T helper 2 cell (TH2) dependent allergic reactions against insect venom. However, therapy has to be followed for 3–5 years and after completion of this period the risk of a systemic reaction remains or may reoccur. Although immunoregulation has been shown to be a major mechanism of action, the exact molecular events that are induced by VIT are not fully understood. Our studies aim at deciphering such relevant mechanism as a detailed understanding of these pathways could offer new possibilities for a more effective and less extensive therapeutic intervention. The molecules TIM-1 on TH2 and its ligand TIM-4 on dendritic cells (DC) have been postulated as critical players in allergic reactions. Whereas their function in airway hypersensitivity and asthma is discussed controversially, little is known about their role in insect venom allergy.

The aim of this investigation was to analyze the effects of VIT on the expression pattern of TIM-molecules on CD4+ T cells (TC) and DC *ex vivo*. Therefore, we examined TIM-1 and TIM-4 expression on mRNA levels in PBMC from allergic donors before and 1–4 weeks after VIT, as well as TIM-1 expression in isolated CD4+ TC. No significant change in gene expression could be determined at the analyzed time points, neither directly after *ex vivo* preparation nor after 4 h of polyclonal *in vitro* stimulation. Flow cytometry, however, revealed reduction of TIM-1 expression on CD4+ TC and diminished TIM-4 expression on plasmacytoid DC (pDC) after VIT compared to protein levels before treatment. For myeloid DC (mDC) our preliminary data shows similar TIM-4 expression before and after venom immunotherapy.

Our results suggest an effect of VIT on the TIM-1 and TIM-4 surface expression on TC and pDC, respectively. A linked impact on functional TIM-1-TIM-4 interaction and TH2 polarization must be the subject of further investigations and could possibly offer options for therapy improvements.

P011

Development of a standardized puls-controlled ergometry test for diagnosing and investigating cholinergic urticaria

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Background: Cholinergic urticaria (CholU) is a common chronic disease in which active or passive warming leads to the development of pinpoint sized wheals, flares and itch. The current guidelines for diagnosing CholU recommend to assess patients for symptoms after they have exercised (stationary bike or treadmill) for 10 min while sweating. This method does not allow for the identification of trigger thresholds, nor is it suited to investigate drivers and modulators of CholU activity under standardized conditions.

Objective: To develop a standardized and reproducible protocol for testing patients for CholU and measuring trigger thresholds, independent of patients' fitness levels.

Method: 10 CholU patients and 10 age and sex matched healthy control subjects underwent puls-controlled incremental ergometry for 30 min (stationary bike) increasing their puls rates by 3 beats every minute. Body core and skin surface temperatures were measured before (baseline) and during ergometry and the onset of sweating (starch-iodine test) and symptoms (wheal and flare type reactions) were recorded.

Results: All CholU patients and healthy controls were able to complete the ergometry protocol. Body and skin temperatures in CholU and HCS increased during puls-controlled exercising and dropped thereafter, without significant differences between the two groups. Times to onset of sweating were also similar in both groups (CholU vs HCS: 19.0 6.0 vs 20.4 4.6 min). All of the CholU patients but none of the healthy controls developed CholU symptoms (itchy wheal and flare type skin reactions) in response to the standardized ergometry protocol. The mean time to symptom onset was 27.5 7.3 min.

Conclusion: We developed a standardized, easy to perform, reproducible and safe method to test for CholU. Our first results suggest that core body and skin temperature regulation as well as time to sweating in response to exercise are normal in CholU patients. Puls-controlled ergometry will allow threshold measurements and in depth studies of drivers and modulators of disease activity and help with the development of novel and better treatments of CholU.

P012 (O20)

Beta arrestin-2 signalling attenuates contact allergic inflammation and modulates chemokine secretion of keratinocytes

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Beta arrestins are ubiquitously expressed adapter proteins that participate in G-protein receptor signalling through receptor desensitization and internalization. They also act as multifunctional adaptor proteins that direct the recruitment, activation and scaffolding of various cytoplasmic signalling complexes including MAPK and non-receptor tyrosine kinases. Beta arrestin-2 is highly expressed in immune cells where it regulates cell motility and chemotaxis. Using beta arrestin-2 deficient mice (βarr2−/−) opposing effects on the inflammatory response have been observed in experimental models for allergic asthma and wound healing. Here we investigated the role of beta arrestin-2 for contact hypersensitivity (CHS) responses to the obligate contact sensitizer DNFB. We found significantly increased allergic ear swelling in beta arrestin-deficient mice compared to wildtype animals. Immunohistological analyses revealed an increased infiltration of neutrophils in inflamed ear tissue of animals lacking beta arrestin-2. Flow cytometric analyses confirmed the enhanced recruitment of Gr1+/CD11b+ immune cells. Experiments with adoptive transfer of sensitized lymphocytes and bone marrow chimeric mice demonstrated a role for beta arrestin-2 in radioresistant skin cells for the regulation of inflammatory responses during the challenge phase of CHS. Higher basal levels of the chemokines CCL2 and CXCL1 and IFNγ-stimulated levels of CCL8 suggest that the deregulated chemokine production in keratinocytes may be responsible for the enhanced recruitment of myeloid cells.

P013 (O03)

Innate immune signals trigger basophil dependent co-factor induced anaphylaxis independent of IgE

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Classical type I allergic reactions are induced by cross linking of FcεRI on mast cell (MC) and basophil surfaces. Binding of antigens to receptor-bound IgE initiates signal transduction and results in degranulation. The release of preformed mediators like histamine elicits the clinical symptoms of anaphylaxis. Alternatively, anaphylaxis can be initiated by IgG antibodies generally requiring higher allergen doses and involving PAF as mediator. It is increasingly recognized that anaphylaxis can be augmented by co- or augmentation factors, best documented for wheat dependent exercise induced anaphylaxis, alcohol consumption and infections. We aimed to analyze how infections augment anaphylaxis. To this end, mice were sensitized with Ovalbumine (OVA) and prior to low dose OVA-challenge pretreated with different pathogen associated molecular patterns (PAMPs) to mimick infection. We could show that this pretreatment of mice triggers full-blown anaphylaxis as measured by significantly decreased core body temperature correlating significant reduction in systolic blood pressure and an increase in serum histamine levels. Further investigations using basophil depleted and mast cell deficient Kitw-sh/w-sh mice surprisingly showed that PAMP driven anaphylaxis is basophil dependent and mast cell independent. In addition to basophil depletion by anti-CD200R3 antibodies, we investigated Mcpt8-Cre mice, which are exclusively basophil deficient in the absence of other alterations due to direct Cre-toxicity. In contrast to C57BL/6 mice, Mcpt8-Cre mice completely failed to develop PAMP dependent anaphylaxis proving that co-factor dependent anaphylaxis depends on basophil function. Importantly, we also found that PAMP dependent anaphylaxis is absent in passively IgE sensitized mice, indicating a role for IgG1. Indeed, analyzing IgE-knock-in mice, in which the exon for IgG1 is substituted by an exon coding for IgE resulting in IgG1 deficiency and IgE overproduction also failed to show PAMP induced anaphylaxis. To investigate how PAMPs act on basophils triggering IgG1 dependent activation, we generated knock-out mice double deficient in the pathogen recognition receptors TLR2 and NOD2 for the analyses of the PAMP Peptidoglycan. In these mice PAMP dependent anaphylaxis was completely absent. In conclusion, we show that PAMPs can orchestrate co-factor triggered anaphylaxis, which is induced through pathogen recognition receptors, mediated by IgG1, and effective through basophils. These data show for the first time an exclusive role of IgE independent but IgG1 dependent and basophil mediated anaphylaxis. This is of major clinical importance for the diagnosis and management in patients with anaphylaxis and may lead to new therapeutic strategies.

P014 (O34)

Basophils and wasp venom immunotherapy: decreased venom-induced activation accompanied by increased expression of immunoglobulin receptors

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Background: Basophils play a hitherto underestimated role in the human immune system. Upon appropriate activation, basophils not only release histamine in the context of anaphylaxis, but also express CD40L and synthesize immune regulatory cytokines, such as IL-4 and IL-13. Through the exclusive production of Th2 cytokines, basophils may be important for development of IgE-mediated allergy by providing early decisive signals for polarization of the immune reaction.

Objective: In broad terms, high-dose allergen immunotherapy adjust a Th2-dominated immune response. Indication and execution of wasp venom immunotherapy (VIT) are highly standardized, the effectiveness of this treatment is >95%. Therefore, we used wasp VIT for investigation of the functional and phenotypic activity of basophils during immunotherapy.

Methods: Circulating basophils were examined in 24 patients with wasp venom allergy eligible for immunotherapy before start of treatment, as well as after 3 days and after 1 month. After pre-incubation (priming) with IL-3 the specific basophil activation marker CD63 was measured by flow cytometry, both after functional IgE-dependent (wasp venom, anti-IgE) and IgE-independent (fMLP) stimulation. By flow cytometric surface receptor quantification two immunoglobulin receptors were monitored at the same time, reflecting the phenotypic activity of basophils.

Results: After 3 months of VIT, a significant reduction of venom-induced IgE-dependent activation of basophils was observed. In contrast, IgE-dependent activation by anti-IgE was unchanged, as well as by stimulation with the bacterial-derived peptide fMLP. In parallel, an increased expression of FcεRI and FcγRII as early as after 3 days VIT was seen, which reached the level of significance after 1 month of VIT.

Conclusions: The striking dissociation between venom-induced and anti-IgE triggered activation of basophils might be indicative for the relative decline of receptor-bound allergen-specific IgE on basophils in the course of VIT. The increase of immunoglobulin receptors, in particular of FcγRII (CD32), which can interfere with the signaling pathways of FcεRI may additionally contribute to the decrease of venom-specific basophil activation.

P015

Identification of IgE-binding epitopes on treatment antibodies: a pilot study

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Background: Hypersensitivity reactions to biologicals seem to be increasing. Particularly infliximab and cetuximab but adalimumab and rituximab as well have been identified as allergenic. Anaphylaxis to cetuximab, a chimeric mouse-human IgG1 monoclonal antibody approved for oncological target therapy, is caused by pre-existing IgE to the disaccharide galactose-[alpha]-1,3-galactose (alpha-GAL). Allergenic epitopes of other therapeutic antibodies are still unknown.

Objective: To identify IgE-binding epitopes on biologicals as a prerequisite for the development of a sensitive and specific assay for IgE-detection before and during target treatment as a measure of risk management.

Patients and methods: 19 patients, aged 36–76, 6 male, 13 female, with rheumatic diseases with infusion reactions to infliximab, adalimumab and etanercept and one cetuximab-allergic patient were included in this prospective investigation. A standardised questionnaire was developed to evaluate their allergy status. A previously developed immunoblot for sIgE to alpha-GAL was performed with cetuximab (Merck Serono) as target antigen. In order to optimize test specificity, alpha-GAL bound to HSA (Dextra Laboratories, Reading) was used for immunoblot. Sera of 12 patients with type I allergy symptoms were additionally investigated with ImmunoCAP for sIgE to alpha-GAL (Platts-Mills, Virginia). All biologicals were used as target antigens under reducing conditions in immunoblot and were additionally analysed for the alpha-GAL epitope and cleaved into Fab and Fc for further epitope analysis.

Results: Seven of 19 patients with exanthema were classified as type alpha-reaction. 12/19 showed immediate type hypersensitivity reactions after infusion: three after adalimumab, three after etanercept and six after infliximab infusion, which was considered as type beta reactions. Their sera were, therefore, further investigated for IgE-reactivity and specificity. Immunoblot with all therapeutic antibodies as well as HSA-alpha-GAL as target antigens was established. Whereas galactose-detecting lectins also bound to other biologicals than cetuximab, an alpha-GAL-specific monoclonal antibody did not reveal a positive signal except on cetuximab. First results of separation experiments in Fab and Fc indicate that the responsible epitopes are located on the Fab regions of therapeutic antibodies. One cetuximab-allergic patient had sIgE to cetuximab, HSA-alpha-GAL (immunoblot) and ImmunoCAP. Another patient showed sIgE to infliximab, a chimeric antibody like cetuximab, but not to HSA-alpha-GAL in immunoblot, indicating the presence of a different IgE-binding epitope on infliximab.

Conclusion: A sensitive and specific method for the detection of anti-alpha-GAL-IgE has been developed providing a valuable diagnostic tool for the investigation of cases of anaphylaxis alpha-GAL-carrying biologicals. First results indicate the presence of further epitopes on biologicals providing tools that may be the basis for risk management of target treatments in the future.

P016

Metal allergens nickel and cobalt facilitate TLR4 homodimerization independently of MD2

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Problem: Roughly 20% of the population in industrialized countries suffers from contact allergies to Ni^{2+} or Co^{2+} released from metal-plated objects such as costume jewellery, medical implants or pigments in hair dyes and ceramics. Although affected individuals face considerable limitations in health, life quality and professional capability, still no specific medication is available and treatment regimens mainly rely on the use of topical corticosteroids. However, corticosteroids can cause serious side effects upon continuous administration, hampering treatment of patients with chronic or work-related disease histories. Recently, the bacterial LPS receptor human Toll-like receptor 4 (hTLR4) has moved into focus of therapeutic considerations as its activation generates the necessary innate immune signal required to initiate allergic skin responses to Ni^{2+}.

Results: Here we evaluated administration of soluble hTLR4 (sTLR4) variants or disruption of hTLR4 dimerisation as potential strategy to interfere with metal-induced inflammation. Using supplementation experiments in hTLR4-deficient primary human keratinocytes we demonstrate that Co^{2+} resembles Ni^{2+} by triggering proinflammatory responses via hTLR4 and its co-receptor MD2. We show that both metal allergens and LPS require hTLR4 dimerisation to induce cytokine expression as evident by the abrogation of responsiveness by alanine mutation of a single asparagine (N433) at the hTLR4 dimerisation interface neighboring the metal binding domain. Unlike LPS, however, Ni^{2+} and Co^{2+} do not require MD2 to induce hTLR4 activation. As a result, administration of sTLR4 to metal-sensitive cells inhibited Ni^{2+} or Co^{2+}-induced cytokine production without affecting LPS-responsiveness.

Impact: Our results raise the option to employ extracellular, metal-chelating sTLR4 to specifically inhibit metal-induced hypersensitivity in a better-tolerated manner. They further warrant caution with therapeutic efforts targeting the predicted metal-binding sites in hTLR4 by small molecules or peptides as this approach is prone to impair hTLR4 dimerisation and bacterial defenses as well.

P017

Immune alterations in birch pollen allergy patients treated with chemically modified allergens (allergoids)

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Recently, we have shown that specific immunotherapy (SIT) with subcutaneous injections of native birch pollen allergen extract is characterized by a dynamic process of immune alterations involving both T cellular and humoral immune responses against the major birch pollen allergen, Bet v 1. The most striking SIT-induced changes were an only transient increase of Bet v 1-specific type 1 regulatory T (Tr1) cells, a loss of Bet v 1-specific T helper (Th) 2 cell reactivity after the first year of SIT, and a continued increase of birch pollen-specific IgG4 antibodies, which are able to block allergen-IgE interaction *in vitro*. Besides native allergens, allergens modified by pre-treatment with formaldehyde or glutaraldehyde, so called allergoids, can be utilized for SIT. Denaturation by aldehydes should lead to alterations of the allergen structure with loss of IgE-binding epitopes but preserved T cell epitopes.

To assess the influence of SIT with allergoid on allergen-specific T cellular and humoral immune responses, peripheral blood of birch pollen-allergic patients was taken at different time points over an observation period of 30 months, including three consecutive birch pollen seasons. Frequencies of Bet v 1-specific IL-5-, IFNgammaand IL-10-producing T cells were determined by means of ELISPOT analysis and CD4+CD25+CD127low/- regulatory T (Treg) cells by flow cytometry, respectively. Furthermore, antibody concentrations of birch pollen-specific IgE, IgA and IgG were assessed by ImmunoCAP.

The frequency of Bet v 1-specific T cells (i.e. Th1, Th2, Tr1) as well as Treg cells remained unaltered throughout the entire 3-year treatment period. Additionally, allergen-specific IgE concentrations were unaffected by SIT, while IgA stayed at very low levels during treatment. In contrast, a significant increase of birch pollen-specific IgG4 antibodies was observed, during the first 18 months of SIT, but not thereafter. These findings indicate that SIT with modified allergoid establishes allergen tolerance and maintenance by immune mechanisms, which differ profoundly from the alterations induced by SIT with native allergens.

P018

Recurring nearly deadly mosquito bites in a patient with mast cell activation syndrome

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Background: Mosquito allergy is an entity that causes severe large or atypical vesicular and even necrotic local allergic reactions at bite sites in some individuals. Systemic reactions including urticaria, angioedema, dyspnea, or hypotension are less common.

Objective: We report a unique case of mast cell activation syndrome and recurring grade IV allergic reactions to mosquito bites.

Methods: To confirm the mosquito allergy we performed skin prick testing with whole-body extract of two mosquito species that are common in middle Europe (Culex pipiens and Aedes communis). Further, CD63 up-regulation in basophils as a measure of basophil activation was determined by flow cytometry after stimulating patient's and control's basophils with whole-body extract of both mosquito species. To show that specific IgE in patient's serum mediated the severe allergic reactions we additionally performed CD63 flow cytometry analysis with IgE-stripped donor basophils that were sensitized by patient's serum and incubated with mosquito whole-body extract. We also looked for specific IgE against mosquito via CAP.

Results: The skin prick test was highly positive for Culex pipiens but not for Aedes communis. Flow cytometry analysis detecting CD63 up-regulation showed activation of basophils for both mosquito species. CAP did not detect specific IgE against mosquito.

Conclusion: We confirmed the patient's mosquito allergy by prick test and flow cytometry and thus identified the first case worldwide with a grade IV allergic reaction to mosquito.

P019

Pollen activate the NLRP3 inflammasome in human keratinocytes

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Following pollen exposure, cytokines and chemokines play an important role in the immunological cascade leading to allergic sensitization and elicitation. Former studies showed that pollen impact the physical barrier of the skin by influencing the expression of adhesion molecules. In this study we expand this data showing an influence of pollen derived mediators on the immunological barrier of human skin by activation of innate components of the immune system – the inflammasome. Recent studies point to an involvement of the NLRP3 inflammasome both in allergic sensitisation by shaping a Th2 response and in skin barrier function influencing skin homeostasis. To expand knowledge in this field we investigated the impact of pollen derived mediators on the inflammasome of human primary keratinocytes (KC). KC were stimulated with aqueous pollen extracts (APE) of birch and ragweed for different time periods. Cell-free supernatants as well as cell lysates were analyzed for Interleukin (IL)-18 and IL-1 beta release. Protein level of active Caspase-1 was determined by Western Blot.

Results revealed that treatment of KC with both birch and ragweed pollen leads to a rapid release of IL-1 beta. Notably, ragweed pollen exhibit a higher potency for the induction of extracellular secretion of IL-1 beta in human keratinocytes compared to birch pollen. An even more distinctive effect could be achieved with both pollen extracts under inflammatory conditions provoked by prestimulation of the keratinocytes with TNF-alpha and IFN-gamma. Furthermore, IL-18 was enhanced in cell lysates of APE stimulated KC. Pollen also lead to the production of active Caspase-1 pointing to a Caspase-1 dependent processing and secretion of IL-1 beta and thus activation of NLRP3 response after pollen exposure.

In summary, our results support the hypothesis that pollen influence the immunological barrier of the skin via the NLRP3 inflammasome of human keratinocytes. Thus, pollen themselves provide the danger signal necessary not only for sensitisation but also for elicitation of inflammatory allergic skin reactions.

P020

Cell-cell contact is essential for IgE-regulating activity of human CD8-positive cytotoxic T lymphocytes *in vitro*

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IgE-production *in vitro* in patients with extrinic atopic dermatitis (AD) / atopy syndrome is controlled in part by CD8pos T cells. Mechanisms remain unclear. Whereas in mice cell-cell contact is essential for this regulatory activity this was not investigated in humans, yet.

Ficoll-isolated peripheral blood mononuclear cells (PBMC) of seven patients with exacerbated extrinsic atopic dermatitis/atopy syndrome were depleted of CD8pos lymphocytes using two purification cycles (MS-columns, anti-CD8 microbeads, Miltenyi Biotec, Bergisch Gladbach) according to the manufacturer's instruction. 5×10^5 cells/well were seeded in 24 well flat bottom plates (Corning, 6.5 mm transwell inserts, 0.4 m pore size, RPMI supplemented with fetal calf serum) and incubated for 10 days at 37°C. Conditions: (i) all PBMC, (ii) PBMC w/o CD8pos cells, (iii) PBMC CD8-depleted and subsequently reconstituted with CD8pos cells, (iv) CD8-depleted PBMC (lower transwell chamber) and CD8pos cells (upper chamber). Cell free supernatants were collected and stored at −80°C until determination of IgE levels using the ImmunoCap100 system (low rage level, Phadia, Freiburg).
Under these experimental conditions, CD8-depletion resulted in elevated IgE-levels as compared to all PBMC in 50% of patients. CD8-reconstitution with cell-cell contact abandoned this elevation confirming earlier findings. In contrast, CD8-reconstitution in the transwell system, i.e. without cell-cell contact, did not influence IgE-production significantly.
Thus, the majority of IgE controlling mechanisms *in vitro* by CD8pos T cells seems to be dependent of T-B cell contact.

P021

Role of CD19^{pos} lymphocytes in human IgE-production *in vitro*

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In vitro, peripheral blood mononuclear cells (PBMC) from patients with extrinsic atopic dermatitis/atopy syndrome produce IgE. Supposedly, CD19^{pos} plasma cells are the main source. Whereas in mice IgE-producing B cells can be killed by CD8^{pos} T cells, regulation of IgE-producers in humans remains unclear.
Heparinised blood of nine adults with exacerbated extrinsic atopic dermatitis / atopy syndrome (IgE > 150 < 15000 kU/l, no immunosuppressive systemic therapy >8 weeks, mean serum IgE-levels 2800 4546 kU/l) were depleted of either C19^{pos} or CD8^{pos} lymphocytes by magnetic beads using two purification cycles following manufacturer's instruction (MS-columns, anti-CD19 or anti-CD8 microbeads, Miltenyi Biotec, Bergisch Gladbach). Cells were incubated in RPMI/10% FCS in 1.8 ml round bottom cryovials for 10 days at 37°C. Conditions: (i) all PBMC, (ii) isolated CD19^{pos} cells, (iii) PBMC depleted of CD19^{pos} cells, (iv) PBMC depleted of CD8^{pos} cells, (v) PBMC CD8-depleted and subsequently reconstituted with CD8^{pos} cells. Purification efficacy was monitored by immuno flow cytometry. Cell free supernatants were collected at different time points and stored at −80°C until determination of IgE levels using the ImmunoCap100 system (low level range, Phadia, Freiburg). Data were expressed in relation to the maximal IgE-level produced by all PBMC (=100%). A two-tailed student t-test was applied for statistical analysis.
IgE-production *in vitro* was detectable in 6 of 9 of patients. In all cases, isolated CD19^{pos} cells did not show any IgE-production. Unexpectedly, supernatants of PBMC depleted of CD19^{pos} cells contained significant elevated IgE-levels as compared to condition (i) and/or (iii).
Thus, under experimental conditions described (i) the source of IgE *in vitro* from atopic PBMC seem not to be CD19pos plasma cells and (ii) within the CD19^{pos} B memory cell fraction IgE-regulatory activity can be postulated.

P022

Hyperreleasability of basophilic granulocytes *in vitro* from patients with atopic dermatitis and hyper-IgE-emia is independent of the activating mechanism

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Lymphocytes and keratinocytes from patients with atopy syndrome are characterized by reduction and hyperreleasability of intracellular granules. Although earlier reports using ELISA suggested that basophilic granulocytes show the same phenomenon, evidence on the cellular level is missing.
EDTA-blood was obtained from six patients with exacerbated atopic dermatitis (AD) and hyper-IgE-syndrome (>2000 kU/l total serum IgE) and from six patients with chronic urticaria (CU) refractory to level III of the international therapeutic guidelines. Systemic therapy was changed to cetirizin. Cells were stimulated with (i) an anti-FceRI antibody, (ii) fMLP, (iii) ionomycin and PMA. Using the extended Flow CAST® kit (Bühlmann, Switzerland)upregulation of CD63 and CD203c on the cell surface was quantified in a FACS-CANTO flow cytometer 15 and 45 min after stimulation. Basophils were identified by CCR3-expression and by granularity in the side scatter and gated.
As compared to CU, patients with AD/hyper-IgE-syndrome showed a significant higher portion of C63- as well as CD203c-expressing basophils following stimulation with anti-FceRI antibody or fMLP after 15 and 45 min ($P < 0.05$–0.01). Ionomycin/PMA resulted in a significant higher portion of CD63/CD203c-expressing basophils in AD-patients after 45 min.
Basophilic granulocytes of atopic patients with hyper-IgE-emia express very high levels of FceRI. Thus, basophil hyperactivation following anti-FceRI antibody stimulation is not surprising. However, granule-hyperreleasability in basophils from patients with AD/hyper-IgE-syndrome was independent of the mode of stimulation pointing to a more basic cell biological alteration. Considering the known granule-defects in lymphocytes and keratinocytes from AD-patients, our data support the hypothesis that granule-hyperreleasability in atopy is a cell type independent phenomenon.

Cellular Biology

P023

High-sulfated hyaluronan prevents TGFbeta-1 induced myofibroblast differentiation by occupation of TGFbeta-1 receptor I binding site

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Myofibroblasts (MFb) are key players in the later stages of wound healing, since they mediate wound closure by wound contraction and synthesis of native extracellular matrix components like collagen I (coll). Fibroblasts differentiate into MFb due to the stimulation with transforming growth factor beta-1 (TGFbeta-1) that *in vivo* is locally released by resident inflammatory cells. The binding of active TGFbeta-1 to its receptor TGFbeta-1RII leads to the recruitment and phosphorylation of TGFbeta-1RI. This ligand-receptor-complex phosphorylates cytoplasmatic Smad2 and Smad3 resulting in the heteromeric Smad2/3 complex that binds to Smad4 and is then translocated to the nucleus to regulate gene transcription. Myofibroblasts are characterized by the expression of the contractile alpha-smooth muscle actin (alphaSMA), coll and the ED-A splice variant of fibronectin (ED-A FN). The MFb mediated formation and contraction of granulation tissue is essential to restore tissue integrity, to reduce the size of the wound and produce a permanent scar. But when apoptosis of MFb is lacking, pathological changes result in hypertrophic scars or fibrosis that furthermore could cause tissue dysfunction and organ failure. Subsequently, controlling MFb differentiation and scar formation is an essential element of wound healing.
Within the scope of testing chemically modified matrix components for their potential use in treatment of wounds we investigate the influence of hyaluronan (HA) and chemically high-sulfated HA (hsHA) on MFb differentiation in the absence and presence of TGFbeta-1. Since sulfated glycosaminoglycans (GAG) are reported to interact with and modulate the bioactivity of growth factors and thereby could modulate wound healing. Hence, primary human dermal fibroblasts (dFb) were exposed to 50 g/ml HA, 50 g/ml hsHA, 10 ng/ml TGFbeta-1 or combinations of the growth factor with HA or hsHA. Before supplementing to dFb, all components were preincubated 1 h at 37°C to allow TGFbeta-1 and HA derivatives to interact in case of combined application. The mRNA expression of alphaSMA, coll(alpha1) and ED-A FN after 72 h was analyzed with qRT-PCR. Furthermore, Smad2/3 translocation to the nucleus was investigated with immunofluorescence staining.
The mRNA expression analysis for alphaSMA, coll(alpha1) and ED-A FN shows a reduction of mRNA levels when TGFbeta-1 stimulated samples were simultaneously treated with hsHA. Furthermore, preincubation of hsHA with TGFbeta-1 impaired the Smad2/3 translocation in dFb while HA did not. The possible molecular mechanism of the inhibition of TFGbeta-1 activity by hsHA was analyzed by *in silico* docking experiments using HA tetrasaccharides. Highly sulfated HA derivatives show significant difference in the distribution on the surface of TGFbeta-1 and furthermore, hsHA derivates bind stronger to TGFbeta-1. In particular, we observed that the higher the sulfation of the HA derivative is, the more probable is that it occupies the TGFbeta-1RI binding site on TGFbeta-1.
Data obtained from cell biological and computational analyses indicate that hsHA hinders dFb to MFb differentiation probably by the occupation of the TGFbeta-1RI binding site on TGFbeta-1 and thus prevents receptor binding, signal transduction and the expression of alphaSMA, coll(alpha1) and ED-A FN mRNA. Thereby application of hsHA could be a therapeutical approach for hypertrophic scars and fibrosis.

P024

Dimethylfumarate impairs lymphangiogenesis by cell cycle arrest

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Different pathologies as lymphedema, cancer, psoriasis or kidney transplantat failure are associated with abnormal lymphatic vessel formation (lymphangiogenesis). There is growing evidence that lymphatic vessels are linked to immune regulation, atherosclerosis, or metabolic diseases. In addition, the lymphatic vessels provide a route for tumor cells to metastasize. Therefore, influencing lymphangiogenesis is a interesting target in various pathological conditions. Recent evidence suggests, that Dimethylfumarate (DMF), known as a highly potent anti-psoriatic agent, might have anti-tumorigenic properties. There are limited data demonstrating that DMF induces apoptosis or enhances the effects of radiation treatment in tumor cells. In addition, it could be recently demonstrated that DMF has anti-angiogenic properties by suppression of the VEGFR-2. We hypothesized that DMF might also have anti-lymphangiogenic qualities. To prove this assumption, we first performed cytotoxicity assays with primary human lymphendothelial cells (LEC). Up to 100 M DMF no relevant LDH release could be demonstrated. In further analysis we could show, that DMF suppresses LEC proliferation in a concentration- and time-dependent manner. In functional analysis (migration- and tube-formation-assays) we could demonstrate a reduced migration rate of LECs as well as an inhibition of the formation of capillary like structures by DMF treatment. To further elucidate wether this anti-lymphangiogenic action is conveyed by an apoptotic mechanism we studied the amount of apoptotic nucleosomes and the activity of caspase 3/7 after DMF treatment. There was no significant apoptosis induced by DMF in human lymphendothelial cells. Therefore, we performed cell cycle FACS analysis demonstrating a pronounced G1 cell cycle arrest. The further evaluation of important cell cycle regulator proteins revealed an increase in p21 and p27 and a suppression of cyclin D1 and A protein expression. The other cell cycle regulators, as for example cyclin E, CDC2 or CDC4, were uninvolved. Interestingly, the superordinated regulator of p21, the tumor suppressor gene product p53, was induced and phosphorylated by DMF treatment. To further analyse the regulation of the important cell cycle regulator p21 we examined its steady state mRNA expression. Here we could demonstrate an increase of p21 mRNA expression. This transcriptional way of regulation was enforced by a posttranscriptional and posttranslational mechanism increasing mRNA- as well as protein-half-life of p21. All these anti-lymphangiogenic mechanisms described, could be conveyed by the suppression of the most relevant pro-lymphangiogenic receptor VEGFR-3, which was downregulated by a posttranscriptional mechanism during the DMF treatment of LECs.
In conclusion, our results provide for the first time clear evidence, that DMF has distinct anti-lymphangiogenic effects. This action seems to be mediated by cell cycle arrest rather than apoptotic mechanisms.

P025 (O12)

Selective blocking of the interaction of extracellular cyclophilins with CD147 reduces recruitment of leukocytes during inflammation

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Leukocyte trafficking and recruitment is a critical process in host immune surveillance, in the defence of pathogens as well as in inflammatory diseases. Extracellular cyclophilins (eCyp) have been identified as a novel class of chemotactic mediators. High levels of eCyp have been detected in inflammatory diseases. Thus, targeting the chemotactic activity of eCyp might be a useful approach to intervene in inflammatory diseases
In the present study, the impact of eCyp for the recruitment of leukocytes during inflammation was analyzed by the generation of a cell-impermeable, non-immunosuppressive CsA derivative, MM284. MM284 inhibits the enzyme activity of CypA like cyclosporine A indicating that it is a tight-binding inhibitor of CypA. The cell-impermeability and its inability to affect calcineurin activity or NF-AT activation are indicative for a lack of T cell mediated immunosuppressive activity of this compound.
The biological effect of MM284 to inhibit eCyp activity was shown by a dose-dependent inhibition of migration of different leukocyte subsets towards CypA. Use of mutant CypA derivates lacking its enzymatic activity revealed that the enzymatic active site of CypA is essential for its chemotactic activity. Inhibition of leukocyte recruitment during inflammation in a mouse model of experimental induced peritonitis and delayed-type-hypersensitivity model by blocking eCyp with MM284 revealed the physiological impact of eCyp in the regulation of inflammation.
Using leukocytes from CD147-deficient mouse we showed that this eCyp-mediated migration of leukocytes is exclusively mediated by CD147. Indeed, CD147−/− leukocytes were unable to migrate towards CypA. Consistently, the extravasation of leukocytes during inflammation was significantly decreased in CD147−/− mice compared to wildtype littermates. Failure of inhibition of migration and inflammation by blocking eCyp by MM284 in CD147−/− mice proved that the action of eCyps in inflammation is exclusively mediated by their interaction with CD147.
Taken together, eCyps might be useful therapeutic targets for reducing inflammatory diseases associated with leukocyte recruitment into tissue. Cell-impermeable and non-immunosuppressive CsA-analogues like MM284 might be good candidates for pre-clinical studies.

P026

Functional biomacromolecules and their properties on human HaCaT keratinocytes in co-culture with bacteria

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Introduction: *In vitro* co-culture systems are practical models to study interactions of antimicrobial agents with human cells and microorganisms. Here, two functional biomacromolecules (FBMs) with different chemical compositions and structures were tested – FBM_E and FBM_T. The scope of FBMs for medical applications seems unlimited as they can be manufactured as nano-coatings to fit wound dressings, medical textiles, cosmetics, or biosensors. However, they need to exert a high antimicrobial activity concomitant with excellent cell compatibility. Hence, we have examined the influence of the FBMs on the viability and proliferation of human HaCaT keratinocytes and the effects on the bacteria *Staphylococcus aureus* and *Klebsiella pneumoniae*.
Materials & methods: Human HaCaT keratinocytes were cultured in keratinocyte growth medium (KGM, Promocell) for 7 days in 75-cm^2 cell culture flaks (Greiner bio-one) at 37°C in a humidified atmosphere containing 5% CO2. *Staphylococcus aureus* ATCC 6538 and *Klebsiella pneumoniae* ATCC 4352 were grown in CASO bouillon (Oxoid) for 24 h at 37°C with gentle shaking. Human cells were harvested through trypsin-EDTA (Invitrogen) treatment and seeded into 96-well micro plates (Greiner bio-one). After 48 h, the human cells were infected with *S. aureus* or *K. pneumoniae* (101 and 102 cfu/ml). After 1 h preincubation, FBM_E or FBM_T with different concentrations were added. After 1, 24 and 48 h, cell viability and proliferation were determined by measuring ATP and protein content. Viable bacteria were quantified by LIVE/DEAD® BacLight(TM) Bacterial Viability Kit (Invitrogen) and BacTiter-Glo(TM) Microbial Cell Viability Assay (Promega).
Results: Infection with bacteria had a distinct negative effect on viability and proliferation of HaCaT keratinocytes. Here, FBMs demonstrated a concentration- and time dependent antibacterial activity. Moreover, the antibacterial efficacy clearly depended on the structure of the FBMs and the bacterial species. While FBM_E was able to kill *S. aureus* at a concentration of 10 g/ml over a period of 48 h, FBM_T only inhibited *S. aureus* growth under the same conditions. In case of *K. pneumoniae* higher concentrations of FBM_E and FBM_T (>30 g/ml) were needed to inhibit bacterial growth. FBMs also showed protective effects on keratinocytes against bacteria.
Conclusions: The *in vitro* co-culture model of HaCaT keratinocytes with bacteria is suitable to analyze the effects of FBMs. It could be demonstrated that human HaCaT keratinocytes in co-culture with bacteria can be protected from bacterial infections by FBMs *in vitro*. Whereas, FBM_E demonstrated a better protection of HaCaT keratinocytes against *S. aureus* than FBM_T did. Moreover, it was observed that the FBMs were more active against gram positive bacteria like *S. aureus* than against gram negative bacteria like *K. pneumoniae*.

P027

Cucurbiturils – supramolecular host molecules with high cytocompatibility

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Introduction: Cucurbiturils are macrocyclic molecules made of glycoluril monomers linked by methylene groups. Because of their outstanding recognition properties in aqueous medium, these pumkin-shaped macrocycles have prompted a rapid development of a diversity of applications e.g. as key units in novel drug carriers and advanced materials. The lack of solubility of hydrophobic drugs in biological medium is a serious impediment to the treatment of various pathologies, including cancer. Due to the hydrophilic carbonylated rims and a hydrophobic cavity, cucurbiturils can complex a wide range of guests and improve their solubility. For dermal applications of cucurbiturils the cytocompatiblility on keratinocytes is of major importance.
Materials and methods: Cucurbiturils were dissolved in DMEM at highest soluble concentrations (CB [5] 15 mg/ml, CB[6] 30 mg/ml, CB[7] 7.5 mg/ml). Proliferation of HaCaT keratinocytes was determined after incubation of the cells with cucurbituril dilutions up to 48 h using a luminometric ATP-assay (ATPlite(TM) M Kit, Perkin Elmer). The ATP dependent light generation was measured with a microplate laser luminometer (LUMIstar Galaxy, BMG LABTECH Ltd.). Two different flow cytometric methods were applied to determine the concentration dependent apoptotic and cytotoxic effects of cucurbiturils after 24 h incubation on HaCaT keratinocytes. Annexin-V/7-amino-actinomycin D (7-AAD) staining was used to detect changes in plasma membrane structure by surface exposure of phosphatidylserin in the course of apoptotic cell death. 7-AAD is a fluorescent DNA-binding agent that penetrates only dead cells. The double staining used in this assay allows differentiation between living, apoptotic, and necrotic cells. Intracellular staining with a monoclonal antibody against active caspase-3, an effector caspase in the caspase cascade during apoptosis, was used to confirm the results. Measurements were done on a BD FACS Canto cytometer.
Results: CB[5] was not cytotoxic on HaCaT keratinocytes over 48 h at concentrations up to 7.5 mg/ml. Only at a concentration of 15 mg/ml cytotoxic effects were detectable after 24 and 48 h. No cytotoxic effects of CB[6] on HaCaT keratinocytes were measured even at the highest soluble concentration of 30 mg/ml over a period of 48 h. For CB[7] cytotoxic effects on HaCaT keratinocytes were detectable at a concentrations $\geq$3.75 mg/ml after 24 and 48 h incubation. No apoptosis was detected for CB[5] and CB[6] at the highest soluble concentrations of 15 and 30 mg/ml, respectively by Annexin-V/7-AAD-staining after 24 h incubation. CB[7] induced an increased level of apoptotic HaCaT cells at concentrations of 3.75 and 7.5 mg/ml after 24 h incubation. These results of the Annexin-V/7-AAD-staining were confirmed by intracellular staining with a monoclonal antibody against active caspase-3.
Conclusions: The cucurbiturils CB[5] and CB[6] exhibited a high cytocompatibility. CB[5] was not cytotoxic at concentrations up to 7.5 mg/ml on HaCaT keratinocytes over 48 h. CB[6] was even better tolerated, no negative effects on cell viability over 48 h were observed at concentrations as high as 30 mg/ml. In contrast, CB[7] was the least cytocompatible cucurbituril with cytotoxic and apoptotic effects on HaCaT keratinocytes at $\geq$3.75 mg/ml after 24 h incubation. In conclusion, due to the excellent cytocompatibility CB[5] and CB[6] offer great potential for dermal biomedical applications.

P028

Ex vivo pathogenicity of anti-laminin gamma 1 antibodies

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Autoimmunity against laminin gamma 1 has been recently described in anti-p200 pemphigoid, an inflammatory autoimmune disease of the skin characterized by severe blistering. Additionally, antibodies against laminin 111 with unclear pathogenic relevance have been reported in various diseases, like lupus erythematosus, pregnancy loss, myocarditis, or Chagas disease. The relevant contribution of anti-laminin gamma 1 antibodies in skin-specific pathology has not yet been documented. We addressed therefore in the current study the pathogenic potential of laminin gamma 1-specific antibodies in previously established *ex vivo* assays. Rabbit antibodies were generated against N- and C-terminus fragments of murine laminin gamma 1. In a first set of experiments, the ability of the antibodies to directly interfere with ligand interactions was assessed in solid phase and cell adhesion assays. Further, the Fc-dependent potential to activate the complement system and inflammatory cells was tested. Antibodies that target fragments of the laminin gamma 1 C-terminus did not interfere with either cell adhesion or integrin binding on laminin, and they were not able to activate the complement cascade or leukocytes. By contrast, the antibodies specific to laminin gamma 1 N-terminus might contribute to tissue damage either by directly inhibiting/disrupting the laminin-nidogen binding or by Fc-mediated activation of inflammatory cells and the complement cascade. These results provide important mechanistic insights into the pathogenesis of tissue damage by laminin gamma 1-specific autoantibodies and greatly facilitate the development of animal models and new therapeutic strategies.

P029

Premature dendritic cells regulate expression of metalloproteinases in dermal fibroblasts

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Dendritic cells (DC) are potent antigen presenting cells. After the capture of antigens in peripheral tissues DC therefore start to mature and migrate to the T-cell areas of draining lymph nodes, where they activate T lymphocytes. While their migration from skin to the lymph nodes DC get in contact with their surrounding microenvironment including several components of the dermal extracellular matrix and stromal cells. It is known that the interaction of DC with their microenvironment is crucial for their ability to migrate and induce immune response. To understand the regulation of DC migration by the dermal microenvironment we focused our work on the interaction of DC and fibroblasts. To imitate the initial activation of DC by pathogens, DC were pre-stimulated for 3 h with LPS. These premature *in vitro* generated human monocyte-derived DC were cocultured with human dermal fibroblasts. Our studies showed that in cocultures the RNA-expression of the matrix metalloproteinases MMP-1, MMP-2 and MMP-3 was significantly up-regulated within 6 h compared to DC or fibroblasts alone. We also observed that compared to the protein-secretion of DC or fibroblasts alone in cocultures the protein-secretion of MMP-1 and MMP-3 was induced and the protein-secretion of MMP-2 was highly up-regulated within 24 h. The comparison of cultures with direct cell-cell-contact or transwell systems showed that soluble mediators from premature DC stimulated MMP expression in fibroblasts. Moreover, IL-1 beta and TNF-alpha were able to stimulate MMP secretion in fibroblasts. Using blocking antibodies and antagonists we showed that TNF-alpha produced by premature DC regulate MMPs expression in fibroblasts.
Taken together premature DC are able to up-regulate the protein-secretion of MMP-1, MMP-2 and MMP-3 from dermal fibroblasts via the secretion of TNF-alpha.

P030

The role of Occludin and ZO-1 in wound healing

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Background: Besides their important role in barrier formation, tight junction (TJ) proteins are known to be involved in proliferation and differentiation. These processes are essential for normal wound healing and impaired in chronic wounds. Therefore we investigated the TJ proteins Occludin (Ocln) and ZO-1 in normal wounds by using an ex-vivo model as well as in tissue samples of chronic wounds. Because increased levels of IL-1beta and TNFalpha are hallmarks of chronic wounds we investigated to what extent these cytokines influenced wound healing as well as TJ protein localization.
Methods: Wound healing progress as well as ZO-1 and Ocln immunostaining were investigated in porcine ex-vivo wound healing models with or without application of TNFalpha, IL-1beta or both cytokines in concentrations present in chronic wounds. The results were compared to immunolocalization of Ocln and ZO-1 in chronic wounds. In addition, we investigated the effect of knock-down of Ocln and ZO-1 by siRNA in cultured keratinocytes in BrdU-, scratch wound-, adhesion-, differentiationand cytokine release-assays.
Results and discussion: Ex-vivo wounds treated with TNFalpha and the combination of TNFalpha and IL-1beta showed significantly decreased healing progress compared to normal wounds.
Occludin, in unwounded skin located almost exclusively in the stratum granulosum, showed the same localization at the wound margins but a broadened distribution in the regenerating epidermis during normal healing. In chronic wounds, Ocln was lost completely in the regenerating epidermis and at some wound edges. Application of the cytokines either alone or in combination caused in some samples the complete loss of Occludin in the regenerating epidermis.
ZO-1 was found in the stratum granulosum and upper stratum spinosum of unwounded skin, but as normal healing progressed, it showed a broadened distribution at the wound edge and in the regenerating epidermis. The same was true in chronic wounds, even though the area of broadened expression was more extended. In addition, some chronic wounds showed a total loss of ZO-1 at the wound margins and in the regenerated epidermis. Treatment with the cytokines led to a similar distribution of ZO-1 in the regenerating epidermis as in chronic wounds, but not at the wound margins.
To further elucidate the putative role of Ocln and ZO-1 in wound healing we knocked-down these proteins in keratinocytes by siRNA. Downregulation of Ocln resulted in increased scratch coverage in scratch assays without changes of proliferation, indicative for accelerated migration. In addition, it reduced cell-cell and cell-matrix adhesion and differentiation. ZO-1 had only a slight impact on cell scratch healing and no influence on proliferation, but affected cytokine release.
In conclusion, our results show that there is a difference in expression and localization of ZO-1 and Ocln between normal and chronic wounds and that elevated levels of IL-1beta and TNFalpha may be partly responsible for these alterations. Because both proteins contribute to cellular characteristics important for wound healing their altered expression is likely to be involved in the pathogenesis of chronic wounds.

P031

Role of individual respiratory chain molecules for fibroblast ageing

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Mitochondrial production of reactive oxygen species (ROS) is suggested to play a key role in organismal ageing. The specific role of mitochondrial pathways in this process is still unclear. We presumed that a dysfunction of parts of the mitochondrial respiratory chain leads to enhanced oxidative stress, ROS production, DNA damage, cellular senescence and ageing. The identification of relevant respiratory chain molecules and pathways activated by these should provide deeper insights into the process of ageing and may point to new therapeutic structures.
We used different mouse strains with defined and stable mutations in mitochondrial genes encoding for respiratory chain proteins of complex I–V and uncoupling protein 2. Primary skin fibroblasts of mutated mice were isolated and exposed to cellular stress exerted by genotoxic agent doxorubicin, UVB irradiation and hydrogen peroxide, respectively.
Analysis of expression and activation of different age-related pathways like p53, p38, JNK, and NF-κB, age-related histones (H3K9 and γH2AX) and β-galactosidase was performed. Mice were analyzed at different time points (0, 3, 6, and 12 months). One of six analyzed strains, C57BL/6J-mtALR/LTJ, with a single polymorphism in NADH dehydrogenase subunit 2 gene in complex I, shows phenotypically markers of ageing such as hair loss and decreased fibroblast growth in contrast to the control strain C57BL/6J-mtAKR/J. Interestingly, this strain showed decreased ROS production after doxorubicin and hydrogen peroxide treatment of isolated fibroblasts. However, immunoblots showed that age-related proteins such phospho-p53, γH2AX, IκBα and β-galactosidase were expressed/activated as early as 2 h after exposure, while other strains showed delayed expression of these molecules after 4–8 h. Based on these findings, it might be suggested that mitochondrial DNA mutation-associated ageing can occur independently of ROS production, but involves histone modification and activation of particular stress signaling pathways.

P032

Kinome profiling of human regulatory T cells reveals novel target structures to modify their functional activity

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Naturally occurring CD4+CD25+ regulatory T cells (nTreg) are essential for T cell homeostasis and the maintenance of peripheral tolerance. They prevent the activation of autoaggressive T cells in the context of autoimmune diseases and suppress inadequate allergen specific T cells. On the opposite, nTreg also inhibit effective immune responses against tumors such as melanoma. Beside a number of nTreg-associated molecules such as Foxp3, CTLA-4 or GARP, thought to play a central role in Treg differentiation and function, several studies suggest the involvement of additional regulatory elements. Herein, kinase activities seem to play an important role in nTreg fine tuning. Nevertheless, our knowledge regarding the complex intracellular signaling pathways controlling phenotype and function of nTreg is still limited and based on single kinase activity research so far. To gain a more comprehensive insight into the pathways determining Treg function we performed kinome profiling of human nTreg at different activation stages compared to CD4+CD25− T effector cells (Teff). The majority of kinases were not exclusively expressed or activated in either nTreg or Teff. However we observed important quantitative differences in both populations. Resting and activated nTreg showed an altered pattern of CD28-dependent components and of kinases involved in cell cycle progression such as CDK2 and cytoskeletal reorganization such as PAK2, also described as a positive regulator of T cell activation that interferes with NFAT expression and IL-2 production. Additionally, significant up-regulation of kinases in activated nTreg but not in Teff such as EGFR, Akt1 or CK2 demonstrate that a specific molecular activation pattern defines the activation state of human nTreg. Taken together, the detailed investigation of kinome profiles that control the functional properties of human nTreg opens the possibility to identify new molecular targets for the development of effective immunotherapies against unwanted T cell responses in allergy, autoimmunity and cancer.

P033

Modulation of cellular functions by artificial extracellular matrices used as biomaterial coatings

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Functionalization of biomaterials has evolved as a promising strategy to improve wound healing and biomaterial integration. The SFB-TR67 focuses on artificial extracellular matrices (aECM) composed of ECM proteins and glycosaminoglycans (GAGs) to be used as implants or coating for biomaterials. Since native ECM provides positional and environmental cues guiding the function of immune and tissue cells aECM coatings are suggested to have strong modulating impacts on cellular responses upon biomaterial implantation. In our approach we test aECM consisting of collagen I and the GAGs hyaluronan (HA) or chondroitin sulfate (CS). The GAGs are chemically modified by the introduction of additional sulfate groups resulting in low-sulfated and high-sulfated GAG derivatives. Within the native ECM sulfate groups of GAGs represent important binding sites of cytokines and growth factors and regulate their distribution and bioactivity. Thus, incorporation of additional sulfate groups to HA and CS is suggested to increase the modulating capacities of aECM coatings by providing additional interaction sites for endogenous mediators.

We could show that aECM containing sulfated HA derivatives have the potential to control adsorption and release of TGF-β1, an important factor for the coordination of wound healing. Artificial ECM composed of sulfated GAGs were found to induce a 'proliferative phenotype' in human dermal fibroblasts (dFb) characterized by accelerated adhesion and proliferation of dFb, but no extensive expression of genes related to extracellular matrix deposition and scarring that is benefiting for cutaneous wound healing. Moreover, aECM containing sulfated HA derivatives dampen the inflammatory cytokine response by macrophages in favour of the release of the anti-inflammatory cytokine IL-10 which represents a crucial step in the transition from the inflammatory to the regenerative phase of wound healing. Furthermore, sulfated GAGs also modify established interactions of fibroblasts and dendritic cells: Fibroblasts grown on aECM with sulfated HA have a decreased potential to stimulate MMP-9 release from dendritic cells thus modifying their potential of cell migration and tissue remodelling during wound healing.

Thus, these aECM might be a useful tool for biomaterial applications to modulate immune responses and support wound healing.

P034

Contribution of tight junction proteins to ion, macromolecule, and water barrier in keratinocytes: no direct effect of claudin-1 on water barrier

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Tight junctions (TJs) are able to form a selective barrier for ions, water, and macromolecules in simple epithelia. In keratinocytes and epidermis, TJs were shown to be involved in individual barrier functions. Absence of the TJ protein claudin-1 (Cldn1) results in a skin barrier defect characterized by a lethal water loss. However, detailed molecular analyses of the various TJ barriers in keratinocytes and the contribution of distinct TJ proteins are missing. Here, we discriminate for the first time TJ-dependent paracellular resistance from transcellular resistance in cultured keratinocytes by using two-path impedance spectroscopy. We show that TJs form a barrier for Na^+, Cl^-, and Ca^{2+}, and contribute to barrier function for water and larger molecules of different size. Increased paracellular permeabilities for ions and larger molecules are found after knockdown of Cldn1, Cldn4, occludin, and ZO-1, showing that all of these TJ proteins contribute to barrier formation. Remarkably, Cldn1 is not indispensable for TJ barrier function for water in cultured keratinocytes, but influences stratum corneum (SC) proteins important for SC water barrier function. In addition, it is crucial for TJ barrier formation for macromolecules with a size of allergens.

P035

Identification of microRNAs differentially regulated during human sebaceous lipogenesis

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MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression post-transcriptionally by interfering with messenger RNAs (mRNAs). miRNAs are considered key regulators of animal development and influence several biological processes. miRNAs also regulate several aspects of the morphogenesis and homeostasis of the skin and its appendages, and miRNA deregulation has been shown to be associated or even causally related to several skin diseases. Despite the importance of sebaceous glands for skin physiology and their involvement in several skin disorders, the role of specific miRNAs in the sebaceous differentiation and the associated lipid synthesis has not been studied systematically so far. Here, we employed the immortalized human sebaceous gland cell line SZ95 to identify specific miRNAs involved in the regulation of sebaceous lipogenesis.

Inhibition of global miRNA activity in human SZ95 sebaceous gland cells was achieved by transfection with siRNAs directed against the DICER transcript, encoding a key enzyme of miRNA biogenesis. Sebaceous lipogenesis was induced in SZ95 sebocytes by addition of linoleic acid (LA) and ciglitazone (CIG) and microarray-based miRNA expression profiles were obtained on an Agilent platform. The expression of selected miRNA candidates was measured by Taqman quantitative real-time RT-PCR. Increased activity of one validated miRNA was attained by transfecting SZ95 sebocytes with miR-574-3p mimics.

Downregulation of sebaceous lipogenesis was detected in DICER-impaired SZ95 sebocytes. Using microarrays, we identified twelve significantly upregulated and nine significantly downregulated miRNAs in LA- and CIG-treated SZ95 sebocytes as compared to non-treated cells. Validation of a subset of miRNA candidates by qRT-PCR confirmed upregulation of miR-203 and miR-574-3p and downregulation of miR-7 during sebaceous lipogenesis. The two upregulated miRNAs have been previously implied in keratinocyte differentiation, whereas the involvement of miR-7 is a novel finding. Increased activity of miR-574-3p augmented lipogenesis in SZ95 sebocytes. In conclusion, global miRNA activity is essential for lipid synthesis in human SZ95 sebocytes. This study also identified several miRNAs that are differentially regulated during human sebaceous lipogenesis. These candidate miRNAs may play a role in the pathophysiology of sebaceous gland-associated diseases.

P036

Low concentrations of curcumin in combination with UVA reduce collagen expression in human skin fibroblasts

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Curcumin is a pharmacologically active substance isolated from the rhizome of the plant *Curcuma longa* Lin. (Zingiberaceae family). The plant is used for thousands of years as a remedy in Asian traditional medicine showing among others anti-inflammatory, anti-oxidative, and anti-tumor activities. The therapeutic benefit of curcumin is hampered by a very low absorption after dermal or oral application. In former studies we could show that the anti-cancer effects of curcumin could be enhanced by the combination of curcumin treatment with light irradiation. Under this condition very low curcumin concentrations (<1 g/ml) were sufficient to induce cell growth arrest and apoptosis.

We do not know if UVA can also enhance the effects of curcumin on dermal cells. Therefore, we investigated in a first approach the effect of curcumin and UVA on dermal fibroblasts focusing on the regulation of collagen synthesis.

Human dermal fibroblasts were pre-treated with low curcumin concentrations for 2 h and irradiated with 1 J/cm UVA. After 24 or 72 h the concentration of pro-collagen-1-N-terminal-peptide (P1NP) and TGF-ß1 was measured in the supernatant. In parallel the incorporation of BrdU in DNA was determined using a BrdU Cell Proliferation ELISA. The toxicity of curcumin and UVA was tested with the Cytotoxicity Detection Kit (LDH).

First, we defined the effective concentration range of curcumin/UVA on cell proliferation in human fibroblasts. We found a strong inhibition of BrdU incorporation in DNA with curcumin concentrations of 0.05–0.3 g/ml combined with UVA irradiation and used this range in all following experiments. Under these conditions curcumin had no toxic effect on dermal fibroblasts. To study the effect of curcumin/UVA on the metabolic activity of fibroblasts we investigated collagen synthesis and the release of TGF-ß1, one of the major autocrine/paracrine regulators of collagen synthesis. After treatment with curcumin and UVA the concentration of newly synthesized collagen decreased concentration dependent. Concomitantly we found a distinct reduction of TGF-ß1 release in a concentration dependent manner. Supplementation of the culture medium with recombinant TGF-ß1 could partly compensate the curcumin/UVA dependent suppression of collagen synthesis indicating a mediating role of TGF-ß1 in the curcumin/UVA induced down-regulation of collagen production.

Our results show that low concentrations of curcumin can diminish collagen expression if the treatment is combined with UVA irradiation. Further investigations will elucidate the exact mode of action and may lead to a new strategy for the therapy of diseases related to overproduction of collagen.

P037

Sebaceous gland size and sebocyte number are dependent upon hair follicle cycle stage in murine skin

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The sebaceous gland forms part of the pilosebaceous unit and is essential for the maintenance of the skin barrier. Besides the production of lipids and their excretion to the skin surface, the sebaceous gland is actively involved in many other processes including hormone metabolism and the production of antimicrobial peptides. It is well established that the hair follicle and the sebaceous gland act co-dependently, with some cutaneous pathologies arising from both hair follicle and sebaceous gland influences, such as scarring alopecia. Changes in the hair follicle cycle coincide with changes in skin thickness, which affects the epidermis, dermis and the subcutis. Here we investigated the effect of the hair follicle cycle on the size of the sebaceous gland and number of mature sebocytes present. Sebaceous gland size and sebocyte number were measured in C57BL/6 mice at time points P1, P8, P17, P19, P21 and P32 post depilation in order to include the first full hair follicle cycle (3 mice per time point, 10 sebaceous glands per mouse). Sebaceous gland size and sebocyte number measurements were taken from sebaceous glands associated with non-tylotrich hair follicles. It was found using one way ANOVA that both sebaceous gland size and sebocyte number changed significantly over the course of the various time points ($P < 0.001$ for both data sets). *Post hoc* analysis (Tukey HSD) showed that both sebaceous gland size and sebocyte number rose significantly during the switch from telogen to mid anagen phase (P1–P8) ($P < 0.001$) by almost double. As the hair follicles progressed through mid anagen to late anagen (P8–P17) sebaceous gland size and sebocyte numbers decreased significantly ($P < 0.001$). There were no changes in sebaceous gland size or sebocyte number through late anagen to late catagen (P17–P21) however, sebaceous gland size and sebocyte number both significantly decreased further as the hair follicles returned to telogen (P21–P32) ($P = 0.01$), with no significant difference between telogen follicles at P1 and telogen follicles at P32. These results suggest that sebaceous gland size and mature sebocyte number change in a cyclical manner which is dependent upon hair follicle cycle stage in murine skin. Future studies analysing number of mature sebocytes or sebaceous gland size within murine skin should consider the stage of hair follicle cycle of the associated hair follicle.

P038 (O04)

Epidermal cFLIP is a critical regulator of skin homeostasis and protects embryonic development in mice

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The control of cell death in the skin is important to maintain the homeostasis of the epidermis. Key proteins within cell death signalling platforms are FADD and the initiator cysteinyl-aspartate specific protease (caspase)-8. Caspase-8 is a close homologue of cellular FLICE-inhibitory protein (cFLIP) with proteolytic activity. Interestingly, mice that constitutively lack any of these three molecules die at early embryonic age. Epidermis-specific deletion of either FADD or caspase-8 resulted in the development of a severe inflammatory skin disorder. This skin disorder was explained by increased programed necrosis (necroptosis) in the epidermis and was fully rescued by additional ablation of the kinase receptor interacting protein (RIP)-3. These data suggested that a major role of caspase-8 in the skin is the inhibition of necroptosis rather than the activation of apoptosis. The role of cFLIP, as regulator of caspase-8, for the control of inflammatory and cell death pathways in the skin is, however, unclear to date. Recent molecular and biochemical studies revealed that intracellular signalling complexes such as the ripoptosome, TNF complex II or the RIG-I complex regulate apoptotic and necroptotic cell death in a cFLIP-dependent manner. In particular in transformed keratinocyte cell lines, these complexes are tightly and differentially regulated by the long or the short isoform of cFLIP.

To further dissect the signalling pathways involved in the control of cell death responses in the skin, we have generated mice lacking cFLIP in the epidermis. Strikingly constitutive epidermal-specific deletion of cFLIP using keratin 14 (K14)-dependent Cre-expression led to embryonic death around E10. To overcome the limitations of embryonic lethality, we next generated mice with inducible postnatal deletion of cFLIP. These mice were fertile and viable in the expected Mendelian ratio. Shortly after topical 4-HT application, cFLIPfl/fl-K14CreERtam mice exhibited severe alteration and macroscopically detectable inflammation of the skin. Histological and immunohistological analysis revealed an increase of dead keratinocytes in the basal layer of the epidermis, whereas control mice did not show any overt phenotype.

Further detailed analyses of these mice and mechanistic studies how loss of cFLIP results in a severe skin phenotype will be presented at the meeting. Our data demonstrate that cFLIP is of critical importance to control survival and inflammatory responses in the skin.

P039

The role of visceral adipose tissue derived serine protease inhibitor in psoriasis

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Psoriasis is a common, obesity-associated, chronic inflammatory skin disease. At present it is not known how obesity is involved mechanistically in the pathogenesis of psoriatic inflammation. Adipokines have recently been implicated – in addition to their key role in the regulation of fat metabolism – as a link between metabolism and immunity/inflammation. In our studies we focused on the adipokine vaspin, a suggested serine protease inhibitor of the serpin family.

In healthy individuals the vaspin serum level correlated with the BMI. However, vaspin serum levels of psoriasis patients were independent of BMI in all subgroup analysis. We identified keratinocytes as the major source of vaspin in skin. Fibroblasts and endothelial cells did not express vaspin. To analyze the regulation of vaspin in keratinocytes, cells were stimulated with different psoriasis relevant factors. IL-6, IL-8, IL-17, IL-22 did not affect vaspin expression. In contrast, TNF-α – one of the main inflammatory effectors in psoriasis – significantly diminished the vaspin expression and secretion by keratinocytes. Since TNF-α is elevated in psoriasis the expression of vaspin in psoriasis was analyzed. Indeed, by comparing healthy, lesional and non-lesional psoriatic skin we showed a reduction of vaspin expression in lesional psoriatic skin by RT-PCR and immunohistochemistry.

Taken together, we identified the adipokine, vaspin, as a potent negative regulator of TNF-α secretion from keratinocytes. Considering that vaspin is a suggested inhibitor of serine proteases and its anti-inflammatory properties we assume that the fine tuned balance of proteases and inhibitors which are involved in the desquamation process as well as in the regulation of inflammation may be disturbed in psoriasis and thus, altered vaspin expression might contribute to the maintenance of psoriasis.

P040

Keratinocyte differentiation from human induced pluripotent stem cells

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Pluripotency has been induced in different human and mouse somatic cell types through the overexpression of specific transcription factors such as Oct4, Klf4, Sox2 and c-myc. These induced pluripotent stem cells (iPSCs) show the same properties as embryonic stem cells e.g. unlimited growth, the ability to differentiate into nearly every cell type and the contribution to germline-competent chimeras in mice.

The application of human induced pluripotent stem cell (hiPSC) technology to regenerative medicine in dermatology would allow the creation of patient-specific and disease-specific stem cell lines and through their differentiation into cutaneous lineages the establishment of organotypic skin cultures.

We have efficiently generated specific hiPSCs from fibroblasts of patients' skin biopsies. These iPSCs are highly similar to human embryonic stem cells. Here we show that these hiPSCs derived from fibroblast can be differentiated efficiently into keratinocytes by treatment with defined medium cocktails. Time course experiments show the expression of different keratinocyte markers at different stages of the differentiation protocol.

Thus generated keratinocytes might be useful in future in regenerative medicine, for the treatment of severely burned patients or for the therapy of genetic skin diseases.

P041 (O36)

Reprogramming of human melanoma cells towards induced pluripotency

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The ectopic transient overexpression of different transcription factors such as Oct4, Klf4, Sox2, and c-Myc is sufficient to reprogram somatic cells into induced pluripotent stem cells (iPSCs). These cells share many features with embryonic stem cells (ESCs). Similarly to ESCs they are able to differentiate into cells of all three germ lines. While epigenetic reprogramming by transcription factors holds great promises for stem cell-based regenerative medicine, the generation of induced pluripotent cancer cells (iPCCs) has become of interest in the oncology field.

Here, we show that similar to their healthy counterparts human melanoma cells can be reprogrammed into iPCCs. These cells reactivate the endogenous loci for pluripotency markers and downregulate typical melanoma markers indicating severe epigenetic modifications.

Our results show that human melanoma cells are susceptible to transcription factor-mediated reprogramming and serve as a tool to study the effects of epigenetic modifications in melanoma.

P042

Pseudomonas aeruginosa-induced RNase 7 expression in keratinocytes is regulated by epidermal growth factor receptor – signaling

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Human skin is able to release a high number of antimicrobial proteins (AMP) such as RNases or beta-defensins for innate immune defense. RNase 7 was identified as a major antimicrobial ribonuclease in skin and exhibits a broad spectrum of antibacterial and antifungal activity against several pathogens. Expression of RNase 7 in keratinocytes is inducible by cytokines and bacteria. Since we identified *Pseudomonas aeruginosa* as a potent inducer of RNase 7 expression in keratinocytes, the aim of the study was to gain insight into the involved signal transduction pathways. To this end, human primary keratinocytes were treated with culture supernatants of *P. aeruginosa* and expression of RNase 7 and other AMP was analysed by ELISA and real-time PCR.

Treatment of the keratinocytes with the broad-spectrum metalloproteinase and tumor necrosis factor-alpha converting enzyme (TACE)-inhibitor marimastat strongly decreased the *P. aeruginosa*-mediated RNase 7 induction. It is well known that TACE (Adam 17) is involved in shedding ligands of the epidermal growth factor receptor (EGFR) such as transforming growth factor-alpha (TGF-alpha). In addition, EGFR ligands such as TGF-alpha and heparin binding epidermal growth factor-like growth factor (HB-EGF) are able to induce RNase 7 expression in keratinocytes. Therefore we hypothesized that the EGFR-pathway may be involved in the induction of RNase 7 by *P. aeruginosa*. To address this hypothesis we pre-incubated keratinocytes with the specific EGFR antibody cetuximab and subsequently stimulated the cells with *P. aeruginosa* in the presence of cetuximab. This revealed a strong reduction of *P. aeruginosa*-mediated RNase 7 gene and protein induction whereas *P. aeruginosa*-mediated induction of the AMP human beta-defensin-2 (hBD-2) was less affected by cetuximab.

These results demonstrate that *P. aeruginosa* releases factor(s) which activate the EGFR leading to the induction of RNase 7 expression. This indicates that the EGFR signal transduction pathway plays a major role in the *P. aeruginosa*-mediated induction of RNase 7.

P043

Characterization of the kallikrein inhibitor mouse Spink6

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Kallikrein related peptidases (KLKs) play an important role in skin homeostasis by controlling the desquamation process. Moreover, they have been implicated in signalling events like activation of proteinase activated receptors, cathelicidin cleavage and IL-1 processing. Their action is therefore tightly regulated. Protease inhibitors like LEKTI, encoded by spink5, are crucial KLKs counterparts in the epidermis as observed in Netherton Syndrome. We have recently identified SPINK6 as a selective KLKs inhibitor in human epidermis and have demonstrated that SPINK6 is transglutaminated in the stratum corneum. In order to get further insight the biology of SPINK6 we studied its mouse homologue. Spink6 is highly conserved among different species. Recombinant mSpink6 exhibited high inhibitory activity in the subnanomolar range against various KLKs including murine KLK5. However, it exhibited some differences to the inhibition of human SPINK6, which might be caused by the altered N-terminus due to an additional exon in the mouse Spink6 gene. Polyclonal antibodies against mSPINK6 exhibited immunostaining at the stratum granulosum and stratum corneum of mouse epidermis and immunostaining in hair follicles, nails and sebaceous glands. Metabolically-induced barrier disruption o mice treated with an essential fatty-acid diet (EFAD mice) and after physical barrier damage (tape-stripping) resulted in decreased expression of Spink6 mRNA expression whereas the immunostaining exhibited no consistent results. Our results identify mouse SPINK6 as a selective inhibitor for KLKs in mouse skin and suggest that SPINK6 is involved in skin barrier function.

P044

Gαi-dependent leukocyte transmigration induces hemorrhage in thrombocytopenia

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Platelets are recognized as effector cells of primary hemostasis. More recently, it was demonstrated that platelets also play a pivotal role in the process of inflammation. In the present study we want to better understand how platelets influence the inflammatory process and investigate their role in the context of inflammation and hemorrhage in two models of cutaneous inflammation. In the inflammatory models of immune complex-mediated vasculitis (ICV) and irritative contact dermatitis (ICD) we observed a significant induction of tissue hemorrhage in the absence of platelets. In addition leukocyte extravasation was reduced. Confirming that leukocyte adhesion is a prerequisite for thrombocytopenic hemorrhage, *in vivo* imaging with the dorsal skinfold chamber (DSC) revealed a local and temporal coincidence of leukocyte vessel wall interaction and initial bleeding. We could show that inflammatory bleeding in thrombocytopenia is prevented in CD18−/− mice, which are deficient in functional β2-integrins. Therefore we tested, if inhibition of sequential steps leading to leukocyte extravasation, may inhibit tissue hemorrhage as well. To analyze if blocking of leukocyte rolling reduces inflammatory hemorrhage, mice were treated with *in vivo* blocking anti-E-selectin antibodies (UZ4) and ICV was performed in thrombocytopenia. We observed reduced leukocyte infiltration and suppressed thrombocytopenic hemorrhage in anti-E-selectin treated mice. To investigate the role of leukocyte recruitment and transmigration for inflammatory bleeding we blocked Gαi-signaling by pertussis toxin (PTX) *in vivo*. In PTX-treated mice (4 g i.v.) infiltrating leukocytes and edema formation were strongly reduced in both inflammatory models, additionally in both models PTX-treated mice were protected from skin bleeding (ICV: PTX 0.94 0.11, control 11.25 1.63 bleeding spots per cm2; ICD: PTX 1.37 0.32, control 5.75 2.35 bleeding spots per cm2). To avoid leukocyte-independent effects of PTX, *ex vivo* treated leukocytes (10 g PTX) were transfused in recipient mice and leukocyte recruitment was evaluated in ICV by *in vivo* imaging. Microscopic examination of recruited leukocytes to the inflammatory site revealed no extravasation of PTX-treated cells up to 4 h observation time. Although transient adhesion of leukocytes was observed, PTX-treated cells did not perform extravasation in the inflamed tissue. In summary, our findings demonstrate that platelets prevent inflammatory hemorrhage while supporting the extravasation of leukocytes and revealed Gαi-mediated leukocyte transmigration as the essential step for the induction of inflammatory hemorrhage.

P045

Von Willebrand factor (VWF) promotes cutaneous leukocyte extravasation

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Von Willebrand factor (VWF), a well-known key player in hemostasis, is increasingly recognized as a proinflammatory protein. Previous studies have shown that VWF is an important regulator of leukocyte recruitment into the inflamed peritoneum. However, the role of VWF for cutaneous inflammation is yet unknown. In this study, immunofluorescent staining revealed massive VWF secretion into inflamed skin of patients suffering from leukocytoclastic vasculitis (LcV) as well as into the inflamed skin of mice. To clarify the role of VWF during cutaneous inflammation, experimental immune complex-mediated vasculitis (ICV) and irritative contact dermatitis (ICD) were performed in mice treated with VWF-blocking antibodies and in VWF−/− mice. We observed a significant VWF-dependent reduction of leukocyte recruitment to the inflamed skin as measured histologically as well as in myeloperoxidase (MPO) activity assays. In the anti-VWF treated group this effect was dose-dependent. In line with these findings, inflammatory edema formation (measured via biopsy wet weight) was significantly decreased. Moreover, *in vivo* imaging in the dorsal skinfold chamber revealed decreased leukocyte adhesion and extravasation after VWF-blockade but unaltered rolling behaviour. To further clarify the VWF-mediated mechanism leading to leukocyte recruitment we performed cutaneous permeability assays. As measured by tissue leakage of Evans blue we observed a significant VWF-dependent reduction of endothelial permeability, thus indicating a direct role of VWF on the endothelium. While peritoneal leukocyte recruitment depends on interaction of VWF with its counter receptor on platelets (GPIbα), there was no mediation of cutaneous inflammation via this pathway. Further we wanted to elucidate whether anti-VWF treatment might be a therapeutic option for the treatment of cutaneous inflammation. To rule out interference with coagulation, we analysed tail bleeding times. While mice deficient in VWF (VWF−/−) or platelet GPIbα (IL4Rα/GPIbα-tg) are known to have prolonged bleeding times, this defect was not observed in mice treated with anti-VWF-antibodies. Importantly, therapeutic treatment with anti-VWF-antibodies leads to significantly reduced cutaneous inflammation. Our data suggest that VWF regulates leukocyte recruitment to the skin by modifying endothelial permeability. Blocking VWF results in decreased cutaneous leukocyte extravasation and provides a novel therapeutic anti-inflammatory approach without interfering with the hemostatic system.

P046

Treatment of autoimmune disease with siRNA targeting p40

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Human inflammatory autoimmune disease like psoriasis and multiple sclerosis but also experimental autoimmune disease in mice are mediated by pro-inflammatory T helper (Th) cells expressing interferon (IFN)-γ (Th1) and interleukin (IL-)17 (Th17). The dendritic cell (DC) cytokines IL-12 and IL-23 are crucially implicated in the pathogenesis of experimental autoimmune encephalomyelitis (EAE). While IL-12 is responsible for the development of Th1 cells, IL-23 promotes the generation of pathogenic Th17 cells. IL-12 and IL-23 are heterodimeric cytokines sharing a common subunit, called p40. Mice deficient for p40 or mice treated with anti-p40 antibodies are protected from encephalomyelitis after active immunization. Antibodies directed against p40 are already established in human psoriasis. Here, we studied a new approach for targeting p40 in autoimmune disease by developing a synthetically-modified small interfering RNA (sm-siRNA) directed against p40. Treating mice with p40 sm-siRNA suppressed IL-12 and IL-23 production *in vivo* and inhibited Th1 and Th17 development after active immunization. This was shown by significant impairment of IFN-γ and IL-17 expression in lymphoid tissue and within the central nervous system in mice which received the p40 sm-siRNA. The clinical course of EAE was silenced by the p40 sm-siRNA administration and protected mice from severe encephalomyelitis. Since the uptake of siRNA by DC is critical and a major technical challenge we analyzed the uptake of the sm-siRNA by DC *in vitro* and *in vivo*. Using flow cytometry and fluorescence microscopy we could demonstrate significant uptake of fluorescence-labeled sm-siRNA by DC *in vitro* without using any transfection reagents. In contrast, minimal uptake by DC was observed *in vitro* when using a non-modified fluorescence-labeled siRNA. The *in vivo* uptake was studied by using radioactive-labeled siRNA. In contrast to non-modified siRNA, sm-siRNA was enriched in CD11c+ DC and biodistribution analysis showed accumulation of sm-siRNA in spleen and lymph nodes. Thus, p40 sm-siRNA provides a novel approach to directly silence IL-12 and IL-23 expression in lymphoid tissue and selectively abolishes Th1/Th17-mediated autoimmune pathology *in vivo*.

P047

Epigen activates a stem cell pool of the developing sebaceous gland and increases sebum production

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The biological functions of epigen (EPGN), the last identified ligand of the epidermal growth factor receptor (EGFR), remain largely unknown. We have previously generated a transgenic mouse line overexpressing EPGN under the control of the ubiquitous chicken–actin promoter (active from E9.5). This resulted in thickened epidermis, enlarged sebaceous glands, and increased sebum levels. Unfortunately, the low fertility of these mice and the ubiquitous overexpression of EPGN from early development precluded detailed studies regarding the mechanism underlying the sebaceous gland enlargement. Interestingly, qRT-PCR analysis revealed increased expression of known hair follicle stem cells in the skin of mice overexpressing EPGN.

To evaluate in more detail the sebaceous gland phenotype of epigen transgenic mice and to find out whether EPGN is able to regulate the hair follicle stem cell pool, we overexpressed EPGN by using a doxycycline-inducible, skin-specific (under the control of the keratin 14 promoter) system. Induction of EPGN expression before epidermal stratification and hair follicle morphogenesis (E11.5) resulted in the already known phenotype, including increased sebaceous gland size, thickened epidermis, and increased cell proliferation in these compartments. The sebaceous gland phenotype fully regressed within a few weeks following doxycycline removal but renewed administration resulted in its re-emergence. However, postponing the induction of EPGN expression to later developmental stages resulted in significantly weaker effects, while induction in adult mice failed to evoke any phenotypical alteration.

When epigen-overexpressing animals were crossed into the EgfrWa5/+ background, a mouse line carrying a dominant negative EGFR, the sebum levels and the size of the sebaceous glands returned to the levels of control animals, indicating that the phenotype is largely EGFR-dependent.

Quantitative RT-PCR revealed increased levels of Lrig1 transcripts in the skin of transgenic mice receiving doxycycline from early embryonic stages, but not in the skin of animals in which transgene expression was initiated only at adulthood. In accordance, immunofluorescence revealed a marked increase in the number of LRIG1-positive cells in the sebaceous gland and the junctional zone of the hair follicles of transgenic animals receiving doxycycline from early embryonic stages. Notably, the increased pool of LRIG1-positive cells was still present even when doxycycline was removed for several weeks. We are currently investigating the expression of other genes encoding known markers of hair follicle stem cells. Our data indicate that early activation of the EGFR by EPGN can expand a stem cell population of the hair follicle and cause hyperplasia of skin compartments that depend on this pool.

P048 (O24)

Casein kinase II regulates the intracellular trafficking of the antigen receptor DEC205 to lysosomal compartments

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The dendritic cell (DC) antigen receptor DEC205 takes antigens into MHC-II+ compartments in DC and mediates effective antigenpresentation. Its intracellular routing is guided by a short 31 amino acid long intracellular domain. This domain contains a putative casein kinase II (CKII) phosphorylation side and we asked whether phosphorylation of DEC205 by CKII is important for the intracellular targeting of DEC205-antigen complexes to deeper endosomal compartments. We generated fusion receptors containing the HuIgG-binding, extracellular domain of human CD16 and the intracellular DEC205 domain (CD16:DEC) and established stably transfected DCEK cell lines. In pulse – chase experiments we incubated the cell lines with HuIgG on ice for 1 h, followed by a chase at 37°C of 30, 60 and 120 min.

We show, that the CD16:DEC transfected cells bind and endocytose aggregated HuIgG, efficiently within 30–60 min, with many vesicles starting to fuse with LAMP-1+ late endosomal compartments. When analyzing the endocytic vesicles in detail, colocalisation of CKII with antigen-loaded vesicles was apparent 30 min after chase, whereas colocalization of CKII with 'unloaded' CD16:DEC+ vesicles did not occur. When we applied CKII specific inhibitors, we observed that surface bound HuIgG remained in vesicles close to the cell surface after a 60–120 min chase period and transport of HuIgG to LAMP-1+ compartments was significantly reduced as compared to untreated controls.

Thus this data show that phosphorylation of the intracellular domain of DEC205 by CKII is crucial for targeting to late endosomal compartments and may have an impact on antigen presentation and generation of T-cell immunity by DC *in vivo*.

P049

Deregulated Akt/mTOR signaling interferes with the balance between keratinocyte proliferation and differentiation and thereby contributes to the pathogenesis of psoriasis

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Psoriasis is a common, highly stigmatising chronic inflammatory skin disease that typically presents with sharply demarked, red scaly plaques that may be painful or itchy. Although biologics directed against certain cytokines, show promising results in the therapy of the disease, a comprehensive understanding of signaling mechanisms that contribute to the pathogenesis of psoriasis is still missing. In a previous study we showed that in healthy skin the PI3K/Akt pathway regulates the equilibrium between proliferation and differentiation and is deregulated in psoriasis. Downstream of Akt the mTOR (mammalian target of rapamycin) cascade is a major integrator of various signals and controls different biological responses such as cell growth, proliferation and differentiation. As psoriatic keratinocytes also show features of perturbed cell growth and differentiation, we asked whether mTOR signalling also plays a role in the pathogenesis of psoriasis. We could show that mTOR and its downstream signalling mediators, the ribosomal protein S6 and 4E-BP are hyperactivated in lesional psoriatic skin compared to non-lesional or healthy skin. While mTOR is activated throughout the whole epidermis, with particularly strong activation in the basal layer, the ribosomal protein S6 and 4E-BP1 are preferentially activated in suprabasal layers of the psoriatic epidermis. We could show *in vitro*, that this activation is induced by psoriatic cytokines such as TNF-alpha and IL-1beta via PI3-K/Akt. PI3-K/Akt activity mainly drives proliferation, while mTOR only partially mediates proliferative responses. At the same time hyperactivation of the pathway through overexpression of Akt blocks keratinocyte differentiation, while blockade of the pathway using either siRNA mediated knockdown or chemical inhibitors favours basal and Ca2+ induced differentiation. Our data suggest that cytokine induced activation of the Akt/mTOR cascade contributes to the induction and/or maintenance of the psoriatic phenotype through the induction of proliferation and blockade of proper differentiation. This points towards mTOR as a potential target for therapeutic intervention in psoriasis and suggests the use of topical formulations of mTOR inhibitors in psoriasis trials, especially as mTOR is efficiently inhibited by rapamycin (sirolimus), which is a pharmacologically well-established drug.

P050

Dimethylfumarate and NF-κB-inhibitors: synergistic effects and implications for future therapies

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Notwithstanding the dacade-long use of fumaric acid esters in the treatment of psoriasis, their mode of action is not fully understood and many patients suffer from considerable and treatment-limiting side effects.

We focused on the activity of dimethylfumarate (DMF) on endothelial cells, lymphocytes and their functional interaction. Given that DMF, as part of its pleiotropic effects, inhibits the central transcription factor NF-κB, we combined it with NF-κB-inhibitors to assess potential synergisms that would allow the use of lower dosages, thus reducing unwanted side effects. Nuclear translocation of NF-κB was partially inhibited at a concentration of 50–100 M of DMF. Downstream, DMF dose-dependently diminished NF-κB-regulated adhesion molecules and inflammatory chemokines, on both mRNA and protein level. As expected, this was accompanied by markedly reduced rolling and adhesion of lymphocytes on human endothelial cells, a key pathogenic step in inflammatory conditions.

Of note, the combination of DMF with two distinct NF-κB inhibitors (KINK-1 and Bortezomib) at low non-toxic doses resulted in profound synergism regarding all parameters measured. Indeed, the respective biological activities on RNA and protein levels were now achieved at concentrations of as low as 5 M DMF. Furthermore, a marked down-regulation of dynamic lymphocyte rolling and adhesion on endothelial cells was detected in comparison to treatment with either substance alone. The combinations showed no significant adverse effect on cell viability.

Overall, our experimental evidence suggests a way to improve the therapy of psoriasis and other DMF-treated conditions through lowered dosages of fumaric acid esters, thus potentially reducing side effects and improving therapy adherence.

P051

Telomerase stimulates RNA polymerase I transcription

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Telomerase is a ribonucleoprotein complex composed of a RNA component (TR or TERC) that serves as template for telomeric DNA synthesis; and the catalytic subunit with reverse transcriptase activity (TERT) which adds telomere repeats to the chromosome ends.

Given the cellular localization of telomerase in the nucleolus and the growing evidence supporting the roles of telomerase in several important cellular processes other than telomere length maintenance, we investigated whether telomerase is involved in transcription by RNA polymerase I.

Using chromatin immunoprecipitation (ChIP) assay, we found that TERT binds to active rDNA genes at promoter and gene internal regions, like RNA polymerase I. This binding seems to be a specific feature of proliferating cells or regenerative tissues.
In addition, we detected telomerase activity associated with to RNA polymerase I, II and III using immunoprecipitation followed by TRAP assay. This finding demonstrates a direct protein-protein interaction between telomerase and the RNA polymerases.
To further investigate the function of telomerase in RNA polymerase I transcription, hTERT was co-transfected with a rDNA reporter plasmid in immortalized fibroblast. Our results show that overexpression of hTERT stimulates RNA polymerase I transcription in a dose-dependent manner. Moreover, telomerase knockdown by transfection with hTERT-shRNA or a dominant-negative telomerase mutant reduces RNA polymerase I transcription in a cancer cell line.
Mouse liver is telomerase positive but there is no binding of telomerase to the rDNA detectable. After partial hepatectomy (PH) 80% of hepatocytes re-enter the cell cycle. ChIP analysis in a time course after PH revealed that during regeneration of the liver, telomerase binds the rDNA, thus implying a post translational modification of telomerase.
Additionally, the transcriptional activity of RNA polymerase I was stimulated after partial hepatectomy, however in a manner independently of the rDNA association pattern exhibited by telomerase.
In an attempt to identify genes bound by telomerase we performed ChIP-sequencing. Data analysis revealed that telomerase binds to different classes of genes.
Taken together, beside its role in telomere maintenance, our results provide a novel function of telomerase in transcription of RNA polymerase I and II.

P052

DNase 2 is essential for DNA degradation in terminally differentiated cells of the sebaceous gland and of the hair follicle isthmus

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Holocrine secretion of sebum involves the breakdown of nuclear DNA in terminally differentiated sebocytes. To investigate the underlying mechanism, we generated a mouse model lacking the acid endonuclease, DNase 2, specifically in keratinocytes and sebocytes. Mice carrying floxed alleles of the Dnase2a gene were crossed with mice expressing the Cre recombinase under the control of the K14 promoter to delete DNase 2 in all tissues derived from K14-positive precursors, including the epidermis and the sebaceous glands. The stratum corneum and sebum derived from the hair surface of wildtype mice contained high levels of acid DNase activity whereas eluates from stratum corneum and hair of DNase 2-deficient mice were virtually free of this activity. While the epidermis of mutant mice appeared normal, the sebaceous glands contained aberrantly large amounts of residual DNA. *In situ* labeling of DNA showed that terminally differentiating DNase 2-deficient sebocytes failed to remove nuclear DNA. In addition, the breakdown of nuclear DNA in the isthmus region of the hair follicle was suppressed by the deletion of DNase 2. These data demonstrate that DNase 2 is essential for the autonomous removal of DNA from terminally differentiated sebocytes and hair isthmus keratinocytes. Thus, our study provides mechanistic insights into the holocrine secretion of sebum and reveals previously unrecognized similarities in the cell death programs of the sebaceous gland and the isthmus of the hair follicle.

P053

Autophagy controls sequestosome-1 in the thymic epithelium and in the epidermis

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Autophagy is lysosomal degradation program of cellular components. Critical functions of autophagy have been identified in multiple tissues, yet the roles of autophagy in the epidermis and other epithelia are largely unknown. Here we have abolished autophagy in keratin K14-expressing epithelia and studied the molecular consequences. Mice carrying floxed alleles of ATG7, an essential regulator of macroautophagy, were crossed with mice expressing the Cre recombinase under the control of the K14 promoter to suppress autophagy in all K14-positive cells and in cells derived from K14-positive precursors, such as thymic epithelial cells. Western blot analysis of LC3 and fluorescence detection of the recombinant green fluorescent protein (GFP)-LC3 reporter protein on autophagosomes showed that thymic epithelial cells and epidermal keratinocytes activate autophagy constitutively. This process was efficiently blocked by deletion of ATG7. Immunofluorescence analysis and Western blot revealed that the suppression of autophagy led to the accumulation of sequestosome 1, also known as p62, in both epithelia. As sequestosome 1 is a multifunctional adaptor protein, autophagy-mediated removal of this protein affects intracellular signaling. Thus, constitutively active autophagy contributes to the control of epithelial homeostasis.

P054 (O11)

Dimethylfumarate impairs neutrophil functions

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Effective host defense against pathogens relies on granulocyte activation. Neutrophilic granulocytes constitute a significant portion of infiltrating cells in different chronic inflammatory diseases, including psoriasis and multiple sclerosis, and fumarates have been shown to improve these diseases. However, the current assumption attributes this effectiveness of fumarates to their modulatory effects on lymphocytes and dendritic cells. In the present study, we addressed the effect of dimethyl fumarate (DMF) on neutrophil functions. *In vitro*, DMF dose-dependently inhibited neutrophil activation, including activation-induced changes of surface marker expression (CD62L, CD11b and CD66b), reactive oxygen species (ROS) production, formation of neutrophil extracellular traps (NETs), and neutrophil migration. Furthermore, their capacity to phagocytise was reduced, and DMF impaired the induction of autoantibody-induced, neutrophil-dependent tissue injury *ex vivo*. To validate these findings *in vivo*, mouse models of epidermolysis bullosa acquisita (EBA), a bullous disease induced by autoantibodies to type VII collagen (COL7), were employed. Anti-COL7 antibodies (IgG) lead to neutrophil-dependent skin tissue injury. Interestingly, in the presence of DMF, induction of skin blistering by anti-COL7 was significantly reduced. Furthermore, DMF treatment of mice with already established immunization-induced EBA resulted in a significant reduction of disease severity. We here demonstrate for the first time that DMF impairs neutrophil functions *in vitro*, *ex vivo* and *in vivo*.

Chemokines/Cytokines

P055

Bovine milk-supplemented medium induces pro-inflammatory cytokines in human fibroblasts *in vitro*

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Bovine milk and products derived thereof are an important component of human nutrition in the Western population. Although, milk is generally associated with a positive image there is ongoing debate if bioactive factors within milk may be harmful to human health. In the context of skin diseases the intake of milk-derived growth factors such as IGF-1 and sex steroids such as testosterone are suspected to contribute to acne vulgaris. In the present study we investigated the impact of commercial bovine milk given to human fibroblasts *in vitro* on liberation of inflammation markers. Our data show a concentration dependent increase of interleukin-6 and -8 measured after 24 h incubation. In parallel we also detected activation of PKB/Akt, p44/42, JNK and p38 by Western blot analysis in response to milk. Future studies utilizing specific inhibitors will test if one or more of the aforementioned signaling molecules are involved in milk-induced release of pro-inflammatory cytokines.

P056

Dimethylfumarate inhibits TNF-α induced expression of atherosclerosis associated cytokines and chemokines in human endothelial cells

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Severe psoriasis is considered to be a chronic systemic inflammatory disease leading to endothelial dysfunction and therefore finally to atherosclerosis. This hypothesis is bolstered by the observation of increased ischaemic heart disease and myocardial infarction in patients with pronounced psoriasis. Dimethylfumarate (DMF) has been used successfully in the therapy of psoriasis vulgaris since 1959. Recent studies provide evidence, that dimethylfumarate has a profound anti-inflammatory action. The knowledge of the effects of DMF on endothelial cells is up to now very sparse. It is well known, that various cytokines and chemokines, as for example IL-8, MCP-1, GMCSF etc., secreted by endothelial cells participate in atherosclerosis. Hence, we hypothesized that DMF suppresses TNF-α induced expression of important atherosclerosis associated cytokines and chemokines and analyzed the underlying mechanisms of regulation. First we could demonstrate a suppression of various TNF-α induced atherosclerosis associated cytokines and chemokines (MCP-1, RANTES, IL-8, PDGF-BB, GMCSF) by Dimethylfumarate in a ECL-based cytokine array in human endothelial cells. These results were verified by ELISA analysis. Here we could demonstrate a time- and concentration-dependent inhibition of the TNF-α induced as well as constitutive expressed cytokines and chemokines by DMF. To analyse the underlying mechanisms of regulation we focused on the expression of the chemokine MCP-1. Steady-state mRNA analysis by RT-PCR demonstrated the suppression of constitutive and TNF-α induced MCP-1 mRNA in a concentration- and time-dependent manner, revealing a transcriptional way of regulation. P65 gene signaling is the most important transcriptional way of regulation of MCP-1 expression. Therefore, we analysed the translocation of phosphorylated p65 into the cell nucleus. Interestingly, DMF suppressed the TNFα induced nuclear translocation of p-p65 in human endothelial cells. These results could be bolstered by the inhibition of p21- and p65-promoter luciferase activity by DMF treatment. Hence, DMF suppresses various atherosclerosis associated chemokines and cytokines in human endothelial cells and therefore might provide an anti-artherosclerotic action, which seems to be conveyed by the inhibition of p65 translocation.

P057

Contrasting, cell type-specific role of ASC in epithelial skin cancer

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An inflammatory microenvironment is considered as a determinant for tumor-progression, but the molecular mechanisms of chronic inflammation promoting tumor-development are largely elusive.
Here, we investigate the dependency of epithelial skin cancer on IL-1 signaling and inflammasome activation. Using the model of two-step DMBA/TPA inflammation-induced skin cancer, IL-1R1−/− and caspase-1−/− mice show a lower tumor incidence, suggesting a role of the inflammasome in carcinogenesis. To dissect the functions of the inflammasome adaptor ASC, which is frequently down regulated in human cancer as a tumor-suppressor versus its role in the inflammatory infiltrate, we generated keratinocyte-specific (ASCf/fK14-Cre+) and myeloid-specific (ASCf/fLysM-Cre+) knockout mice. In a model of chemically induced epithelial skin cancer, ASCf/fLysM-Cre+ showed significantly reduced, while ASCf/fK14-Cre+ displayed a higher tumor-incidence. Subsequently, we identified ASC as a regulator of keratinocyte proliferation.
By further demonstrating ASC loss in human cutaneous squamous cell carcinoma, our results implicate contrasting, cell type-specific functions of ASC as a tumor promoter and a suppressor in skin cancer both in murine and human squamous cell carcinoma.
This study therefore demonstrates how one single protein depending on its tissue expression can diametrically influence tumor growth either via its tumor-suppressive function in tumor-cells or via its pro-inflammatory role in the tumor infiltrating myeloid cells.

P058

IL-31 affects the formation of the mechanical skin barrier and strengthens the antimicrobial defense of the skin partially by upregulating IL-1alpha expression

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IL-31 is an IL-6 type cytokine that is suggested to be an important mediator in allergic skin diseases like atopic dermatitis (AD) and allergic contact dermatitis. IL-31 is highly expressed in the skin of AD patients but its functional role is only poorly understood. We recently demonstrated that IL-31 has the capacity to interfere with the differentiation process of keratinocytes and inhibits the expression of important structural proteins including filaggrin.
In this study we demonstrate that the IL-31 mediated defects in keratinocyte differentiation weaken the mechanical skin barrier allowing an easier transepidermal penetration by allergens and pathogens as well as skin irritating agents in 3D organotypic skin models. IL-31 also has an indirect effect on the processing of filaggrin and the formation of corneosdesmosomes and several desmosomal adhesion molecules by negatively regulating important mediators like Casp14 and KLK7. Thus IL-31 might directly be involved in the pathological weakening of the skin barrier in AD patients leading to increased vulnerability to skin irritants and higher risk for superinfection and allergic sensitization to environmental allergens.
Furthermore, IL-31 was found to have an impact on the expression of components of the IL-1 network (most remarkably IL-1alpha), and antimicrobial peptides like S100A7 (Psoriasin), S100A8, S100A9 and human beta defensin-2 (HBD-2). Both, IL-31 and IL-1alpha upregulate the expression of S100A7 and HBD-2 in 3D organotypic skin models and thus IL-31 can strengthen, either directly and/or by modulating the inflammatory environment, the antimicrobial defense of the skin.

P059 (O35)

The biologic effect of IL-36 on skin cells

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IL-36 cytokines belong to the IL-1 family. We have previously shown that psoriatic keratinocytes show an intrinsically increased expression of IL-36 upon stimulation with IL-17. A lack-of-function mutation of the IL-36 receptor antagonist has been found in patients with severe generalised pustular psoriasis. Recent work has shown that truncated forms of the protein show increased binding affinity to the receptor and higher luciferase activity in IL-8 reporter assays performed in Jurkat cells.
It was the aim of this study to elucidate the functional effects of IL-36 molecules in primary human keratinocytes and fibroblasts. The latter cell type is recognised to be very responsive to IL-1 family members.
Full length IL-36 failed to induce any pro-inflammatory response in fibroblasts when concentrations of up to 100 ng/ml were used. By contrast, an inhibitory trend was observable for the secretion of IL-6, IL-8 and MCP-1 by dermal fibroblasts stimulated with the full length molecule.
However, truncated versions of IL-36 were very efficient in inducing high levels of these mediators with, in particular, a marked effect on IL-8 release. Fibroblasts (but not keratinocytes) derived from psoriasis patients showed a higher sensitivity to stimulation with truncated IL-36 forms with regard to IL-8 release than those from healthy donors.
These findings are interesting in the context of our preliminary results that mechanical stress may increase the expression of IL-36. IL-36 may be an important molecule to link known exogenous trigger factors of psoriasis with increased neutrophil recruitment and thus pustular disease phenotypes.

P060

Th1 and Th2 cytokines modulate differentiation and differentiation-related calcium channels in keratinocytes

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Cytokines play a pivotal role in the pathogenesis of psoriasis and atopic dermatitis (AD). Both diseases are characterised by disturbed maturation of keratinocytes. The regulation of intracellular calcium via the calcium gradient within the epidermis which is crucial for keratinocyte differentiation seems to be defective in AD and psoriasis. However, the interplay between cytokines and disturbed calcium regulation is not clear. To elucidate the link between altered cytokine levels and defective differentiation, we investigated the effects of interleukin-4 (IL4), interleukin-13 (IL13), interferon-gamma (IFNgamma) and tumor necrosis factor-alpha (TNFalpha)on differentiation and intracellular calcium concentration using HaCaT keratinocytes.
We conducted calcium imaging experiments to investigate cytokine effects on intracellular calcium. Stimulating HaCaT cells with the respective cytokine, only IFNgamma induced a calcium influx. IFNgamma-induced calcium-influx was only evoked in the presence of extracellular calcium indicating that IFNgamma induces a calcium-influx across the plasma membrane. Identification of the ion channel is currently under investigation.
To confirm cytokine effects on calcium-induced differentiation, HaCaT cells were treated with cytokines. Transglutaminase 1 (TGM1) mRNA levels were quantified by polymerase chain reaction. IFNgamma increased TGM1 mRNA levels after 24 h dose-dependently. Data from haematoxylin-eosin-staining also showed a strong differentiating effect in the presence of IFNgamma further supporting our results.
To explore long-term effects on proliferating and differentiating keratinocytes, HaCaT cells were treated with a cytokine or a cytokine combination in the presence of 0.07 or 2 mM calcium. Calcium-induced calcium-influx was assessed. IL4 1 ng/ml and IL13 1 ng/ml enhanced calcium influx in proliferating cells after 24 h whereas IL4 30 ng/ml reduced it. We also tested cytokine effects on TRPC6 channels using hyperforin. After 24 h treatment with IL4 or IL13 in various concentrations, hyperforin-induced calcium influx was reduced in differentiating cells. IL13 also decreased calcium influx in proliferating keratinocytes. Data on mRNA and protein level complete our findings.
In conclusion, our data indicate a direct cytokine effect on differentiation and ion channels linked to keratinocyte differentiation.

P061

Distinct contributions of cytokines to autoantibody-induced tissue injury

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Cytokines are pivotal regulators of immune functions. While an integral part of the host response, aberrant cytokine responses are linked to pathogenesis of chronic inflammatory diseases. Consequently, cytokines were successfully identified as therapeutic targets for different chronic inflammatory diseases. Despite aberrant cytokine expression, cytokine-targeting therapies have not been established in autoimmune bullous dermatoses (AIBD), most likely due to lacking functional data or redundant functions within the cytokine family. AIBD are collectively characterized by mucocutaneous tissue injury, autoantibodies against structural proteins of the skin, limited therapeutic options, and an increased mortality. We systematically analyzed the contribution of cytokines to the pathogenesis of autoantibody-induced tissue injury in an animal model of a prototypic AIBD, namely epidermolysis bullosa acquisita (EBA). In experimental EBA, 10 of 23 tested serum cytokine levels correlated with disease activity. Functional analysis revealed a complex and unexpected contribution of these differently expressed cytokines to the pathogenesis: As expected by correlating serum levels, inhibition of IL-1 or GM-CSF significantly reduced skin blistering and/or was effective in a therapeutic setting. However, while TNFα and MIP-1α serum levels correlated with disease activity, blockade of these pathways had little effects on the skin disease. Most interestingly, and in sharp contrast to rheumatoid arthritis, inhibition of IL-6 functions led to enhanced blistering, while treatment with recombinant IL-6 almost completely protected from disease. At the molecular level, IL-6 led to increased IL-1ra concentrations in serum and skin and to protection from apoptosis and proteolysis in the skin. In summary, we provide new insights into the complex cytokine network regulating autoantibody-induced tissue injury, which will facilitate selection of novel therapeutic targets for patients with EBA and other AIBD.

Clinical Research

P062

Treatment of chronic venous leg ulcers with a hand-held DBD plasma generator (PlasmaDerm® VU-2010): results of a monocentric, two-armed, open, randomized, and controlled trial (NCT01415622)

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Plasma (ionized air) comprises a novel treatment modality. We conducted a first clinical trial with the PlasmaDerm® VU-2010 device to assess safety and, as secondary endpoints, efficacy and applicability of 45 s/cm^2 plasma application as add-on-therapy in the treatment of chronic venous leg ulcers. From 4/2011 to 4/2012 fourteen patients were randomized to receive a standardized modern wound care ($n = 7$) or plasma in addition to standard care ($n = 7$) 3×/week for 8 weeks. The ulcer size was determined weekly (Visitrak, photodocumentation). Bacterial species (bacterial swabs), bacterial load (contact agar plates), and pain during and between treatments (visual analogue scale) were assessed at every visit. Patients and doctors rated the applicability of plasma according to a four-item questionnaire. We found that plasma treatment is safe with two SAEs ($P = 0.77$) and 77 AEs ($P = 1.0$) equally distributed among both groups (two AEs probably related to plasma). Plasma treatment resulted in a significant reduction of lesional bacterial load (growth on contact agar plates before and after plasma treatment; $P = 0.03$). A more than 50% ulcer size reduction was noted in 5/7 and 4/7 patients in the standard and plasma groups, respectively ($P = 0.42$). Healing appeared faster in the plasma group and the only patient whose ulcer closed after 7 weeks received plasma. Patients in the plasma group quoted less pain. The plasma applicability was not rated inferior to standard wound care ($P = 1.0$). Physicians would recommend ($P = 0.06$) or repeat ($P = 0.08$) plasma treatment by trend. We conclude that larger controlled trails are warranted with the safe plasma technology.

P063

Development and validation of the Angioedema Activity Score (AAS)

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Background: Recurrent angioedema is a frequent clinical problem characterized by suddenly occurring cutaneous and/or mucosal swellings. It is either mast cell-mediator induced, e.g. in patients with chronic spontaneous urticaria (csU), bradykinin-mediated, e.g. in patients with hereditary angioedema due to C1-inhibitor deficiency or defects, or idiopathic. Depending on their location angioedema may be painful, hindering, disfiguring or even life-threatening. The assessment of disease activity in affected patients is important to guide treatment decisions. Currently, however, there is no standardized and validated outcome measure available to do so. Objective: To develop and validate the first specific patient reported instrument to assess disease activity in recurrent angioedema patients, the Angioedema Activity Score (AAS).
Methods: After a set of potential AAS items was developed, item evaluation and reduction were performed by means of impact analysis, factor analysis, regression analysis and by checking for face validity. In addition, the final AAS questionnaire was tested for its validity and reliability during a 12-week validation study.
Results: In total, data of 110 and 80 angioedema patients were used during the AAS item evaluation and validation phase, respectively. The resulting AAS consisted of five items and was found to have an one-dimensional structure and excellent internal consistency. It correlated well with other measures of disease activity (number of angioedema affected days, patients global assessments) and quality of life impairment [Angioedema Quality of Life Questionnaire (AE-QoL) results, SF-36 scores], thus demonstrating its convergent validity. In addition, the known groups validity and test-retest-reliability of the AAS were found to be good.
Conclusions: The AAS is the first validated and reliable tool to determine disease activity in recurrent angioedema patients and it may serve as a valuable instrument in future clinical studies and routine patient care.

P064

Underlying causes of chronic spontaneous urticaria can not be predicted based on age or gender of patients or clinical characteristics such as activity, duration or impact of disease

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Background: Chronic spontaneous urticaria (csU) is a frequent skin disorder that causes major quality of life impairment and considerable health-care costs. The current EAACI/GA2LEN/EDF/WAO guidelines recommend identifying and treating the underlying causes of csU if possible. This is widely held to be difficult and unsuccessful in most cases.
Methods: 250 consecutive adult csU patients were subjected to a comprehensive diagnostic programme aimed at the identification of underlying causes.
Results: Underlying causes of csU were identified in 176 (70%) of 250 csU patients. The most frequent cause was intolerance to food components (38%), followed by infections (26%) and autoreactivity (20%). Notably, infections were found in 134 subjects (54%), but these proved to be relevant (>50% improvement of csU after eradication) in only 66 patients. In the majority of all patients included (140 subjects), only one single cause of csU was detectable while the existence of multiple causes was less frequent (only 36 patients affected). Although the clinical profile showed some differences related to the cause categories, age, gender, disease activity, duration and quality of life impairment were not found to be suitable predictors for individual underlying causes.
Conclusions: Underlying causes can be identified in a considerable proportion of csU patients. This allows for a specific rather than a symptomatic treatment approach and, thus, for a reduction of disease burden and direct as well as indirect health-care costs. While most patients can be clearly allocated to only one single cause category, thus suggesting the existence of distinct csU subpopulations, the clinical profile does not help to predict individual underlying causes.

P065

High-dose bilastine effectively reduces temperature thresholds in cold contact urticaria

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Background: Cold contact urticaria (CCU) is characterized by itchy wheal and flare responses due to the release of histamine and other pro-inflammatory mediators after exposure to cold. The treatment of choice in CCU is non-sedating antihistamines. However, symptoms of many patients are not sufficiently controlled with standard doses of antihistamines.
Objective: To assess the effects of a standard 20 mg dose and up-dosing to 40 and 80 mg of bilastine in reducing the symptoms of CCU and inflammatory mediator release following cold challenge.
Methods: Twenty CCU patients were included in this randomized, crossover, double-blind, placebo-controlled 12-week study and received placebo, 20, 40 or 80 mg of bilastine daily each for 7 days with 14-day washout periods. The primary readout was change in critical temperature thresholds (CTT). Secondary readouts were changes in pruritus, levels of histamine, IL-6, IL-8 and TNF-alpha collected by skin microdialysis and safety and tolerability of bilastine.
Results: Bilastine 20 mg was highly effective ($P < 0.0001$) in reducing CTT. Up-dosing to 80 mg significantly ($P < 0.04$) increased its effectiveness. At this dose 19/20 (95%) patients responded to

treatment with 12/20 (60%) becoming symptom free. Only one patient was refractory to treatment. Microdialysis levels of histamine, IL-6 and IL-8 assessed 1–3 h after cold challenge were significantly ($P < 0.05$) decreased following up-dosing with 80 mg bilastine. Bilastine treatment was well-tolerated without evidence of increased sedation with dose escalation.
Conclusions: Bilastine was effective in reducing the clinical symptoms of CCU patients. Our study showed that up-dosing of bilastine is safe and supports the guidelines to increase antihistamine doses in urticaria patients not responding to standard doses. The superior effects of high doses of bilastine versus standard doses may be mediated via additional anti-inflammatory effects.

P066

A 16-week open-label study on the efficacy and safety of canakinumab treatment in urticarial vasculitis

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Background: Urticarial vasculitis (UV) is characterized by persistent urticarial lesions (>24 h) combined with histopathologic findings of leukocytoclastic vasculitis. In contrast to chronic urticaria antihistamines as well as immunomodulating therapies show only limited efficacy. Also, none of these drugs are approved for the treatment of UV. Recently, the IL-1 blocker anakinra has been reported to improve symptoms in a patient with therapy-refractory UV.
Objectives: To assess the efficacy and safety of canakinumab, a monoclonal antibody that neutralizes IL-1©, in patients with active, therapy-refractory UV.
Methods: Ten adult UV patients (f/m = 9/1; mean age 53.3 ± 13.0 years) were treated with single 300 mg s.c. canakinumab injections in a 16-week open label trial. Changes in patient-reported mean UV activity scores (UVAS) following canakinumab administration served as primary endpoint. Secondary endpoints included changes in the global assessment of disease activity, changes in acute phase reactants CRP and ESR, serum cytokine levels (IL-1Ra, IL-6) and quality of life during the trial.
Results: Following canakinumab treatment mean UVAS values significantly decreased as compared to baseline ($P = 0.005$). Significant improvement was observed for all efficacy measures including both physician ($P = 0.001$) and patient ($P = 0.05$) global assessment of disease activity, the CRP and ESR ($P = 0.05$). In addition, canakinumab treatment was associated with marked reductions of cytokine levels ($P = 0.05$) and improved QoL ($P = 0.05$). There were no serious adverse events reported, and canakinumab was well tolerated without concern for specific organ toxicity.
Conclusion: In this pilot study, canakinumab showed to be effective and safe and may be considered as a therapeutic option in the treatment of refractory UV.

P067

Bacterial colonization in hidradenitis suppurativa/acne inversa: a prospective study on 50 patients

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Hidradenitis suppurativa/acne inversa (HS) is a chronic, inflammatory, recurrent, debilitating skin disease of the terminal hair follicle, usually manifesting after puberty with deep-seated, painful and inflamed lesions of the apocrine gland bearing areas of the body, most commonly the axillae, inguinal and anogenital regions (Dessauer definition). Its pathophysiology is based on follicular occlusion, while genetic factors, smoking, obesity, mechanical stress, microbial colonization of the lesions and aberrant immune response are associated with its pathogenesis. In order to shed light upon the role of bacteria in the genesis or development of HS lesions, a prospective study was conducted on 50 HS patients. Swab tests were performed from HS lesions of the inguinal region (39%), the axilla (27%), the perianal region (17%), the sub-mammary region (14%) and other rarely affected areas such as the head and the umbilicus (3%). The swab tests were especially selected to guarantee optimal maintenance and viability of both aerobic and anaerobic bacteria prior to their transport on petri dishes for culture. A variety of microbial species were isolated, including coagulase positive and negative Staphylococci, species of the Enterobacteriaceae family, anaerobic species of the non-Enterobacteriaceae, Streptococci and Enterococci. The number and type of bacteria was correlated with the stage of the disease (Hurley) and the localization. *Staphylococcus aureus*, *Prevotella* species and *E. coli* were the predominant species isolated. Interestingly, many species were not detected only on the expected areas, for example anaerobic Enterococci were also isolated from the sub-mammary region. The large variety of bacteria isolated from HS lesions arises the question of the clinical significance of bacterial colonization in HS.The authors Aikaterini I. Liakou and Georgios D. Nikolakis contributed equally to the study.

P068

Platelet-activating factor (PAF) induces wheal and flare skin reactions independent of mast cell degranulation

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Platelet-activating factor (PAF) is a potent mediator of allergic reactions and has been claimed to contribute to the pathogenesis of urticaria, but its role and relevance remain to be characterized in detail.
PAF, histamine, codeine, and saline were injected intracutaneously in 14 healthy volunteers. Wheal-and-flare responses were assessed by planimetric morphometry. Histamine, prostaglandin D2 (PGD2) and PAF levels were measured by skin microdialysis.
Intracutaneous injections of PAF, histamine and codeine resulted in typical wheal-and-flare type reactions. A significant increase in intradermal histamine and PGD2 levels as compared to saline was observed after codeine injection. Following PAF injection, histamine levels and also PGD2 levels did not increase as compared to saline. Also, no significant increase in intradermal PAF levels after injection of histamine, codeine or PAF could be observed
Our findings demonstrate that intradermal PAF injections result in wheal-and-flare type skin reactions without inducing mast cell degranulation. PAF may, therefore, be a relevant mediator in the pathogenesis of urticaria that acts downstream of mast cell activation and independent of H1 receptor activation.

P069

Rupatadine treatment improves quality of life in patients with mastocytosis: a randomized, double-blind, placebo-controlled trial

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The vast majority of adult patients with mastocytosis suffer from cutaneous or indolent systemic mastocytosis, which is associated with symptoms resulting from mast cell mediator release, including pruritus, flushing, diarrhoea and abdominal pain, up to severe and life-threatening anaphylaxis. Therefore, many patients require continuous and emergency medications. Non-sedating antihistamines (nsAHs) are the first line therapeutic strategy to treat mediator-related symptoms. However, data on the therapeutic effects of nsAH therapy in mastocytosis from controlled clinical trials are missing. Therefore, Rupatadine, a nsAH with PAF antagonistic action, was tested for efficacy in the treatment of cutaneous and indolent systemic mastocytosis. A, randomized, double-blind, placebo-controlled, cross-over trial was performed to assess the efficacy of 20 mg rupatadine in the treatment of mastocytosis symptoms. Thirty adult patients were enrolled in the study and effects were evaluated before and at the end of a 28 day treatment period. Patients documented their symptoms by diary and visual analog scale (VAS). Quality of life impairment was assessed by a symptom specific questionnaire for pruritus (ItchyQoL). Rupatadine treatment, but not placebo, resulted in significant quality of life improvement. Patients treated with rupatadine exhibited significantly reduced symptom severity assessed by patient diary and VAS, whereas placebo failed to improve symptoms. These data, for the first time, demonstrate the effects of nsAH treatment in mastocytosis in a randomized placebo-controlled trial. Rupatadine was found to be well tolerated and to control skin symptoms, and improve quality of life in mastocytosis patients. Rupatadine, therefore, can be considered as a first line treatment option for symptomatic patients with cutaneous or indolent systemic mastocytosis.

P070

Assessment of the influence of skin care products on age-associated skin quality and stress reaction

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Introduction: Skin alterations throughout life mirrors whole body aging. Dryness, roughness and changes of surface texture are major clinical markers of aging skin, which also correlates with changes in the composition of sebum as well as a disturbed response to stress. To evaluate the latter process the effect of skin aging on its reaction to mechanical stress was studied in different age groups. The composition of the superficial skin lipids was evaluated before and at the end of the study. In addition, the effectiveness of skin care products in normalizing the signs of skin stress and their influence on skin quality was studied.
Patients and methods: Skin quality parameterers were measured (hydration level of stratum corneum, transepidermal water loss, skin pH and erythema level) and skin mechanical stress was evaluated (18× stripping with D-squame tapes) at the forearms of two groups of healthy individuals younger than 35 years and older than 65 years. Measurements were performed at baseline and 1 h, 6 h, 24 h and 7 days after administration of mechanical stress. Three different 4 cm^2 areas of the stressed skin were treated daily with three different skin care products. A forth area was left untreated. The superficial skin lipid composition was evaluated at base line and at the end of the study for each of the tested areas.
Results: The aged subjects' skin exhibited higher pH level, stronger erythema and higher hydration of the stratum corneum in comparison to young subjects' skin. Young and aged subjects reacted differently to stress. The aged subjects showed a delayed erythema reaction, in comparison with the young skin. The application of topical products – but not all – were able to diminish the skin reaction to stress in young but not in old individuals and one product even slowed down the normal repair process and worsened skin quality. The superficial lipid composition varied significantly between the two age groups and under the application of different skin care products.
Conclusion: Young and aged skin differ in pH level, basal erythema and hydration. They respond to stress in a different manner, which in turn is influenced with skin care products. Certain skin care products – but not all – can normalize the skin reaction to stress and improve the skin quality measurements but others can even worsen skin quality, having a negative influence on the skin. The influence could be effectively assessed by evaluation of skin quality measurements and superficial lipid analysis.

P071

A randomized, double-blind, vehicle-controlled study of diclofenac 3% in hyaluronic acid 2.5% gel for the treatment of actinic keratoses

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Background: The risk for progression of actinic keratoses (AK) to invasive SCC highlights the need for effective and well tolerated management.
Objectives: This is a randomized, double-blind, vehicle-controlled study to assess the efficacy and safety of diclofenac 3% in hyaluronic acid 2.5% gel (DHA) for the treatment of AK, in Greece.
Methods: Written informed consent was obtained from enrolled patients. One hundred patients with AK were randomized to two treatment groups, either to receive treatment with either DHA or vehicle, twice daily for 90 days. Patients were evaluated monthly up to 120 days (follow-up) for efficacy, safety and tolerability.
Results: 71 patients completed the study (placebo group, $n = 38$ and DHA, $n = 33$). At 90 days, 24% ($n = 8$) of patients treated with DHA achieved complete clearance [Target Lesion Number Score (TLNS) = 0, Cumulative Lesion Number Score (CLNS) = 0] compared to placebo ($P = 0.0372$). At 120 days (follow-up), of the DHA treated patients, 42% ($n = 14$) had complete clearance compared to 8% with placebo ($P < 0.001$) and 52% achieved ≥75% lesion clearance from baseline, compared to placebo ($P = 0.0074$). There were not statistically significant differences in the occurrence of adverse events between the two groups and DHA gel was well tolerated. One patient (2.6%) in the active treatment group reported adverse events (dermatitis). Compliance to treatment was excellent for both groups (91% for DHA group vs 87% for placebo).
Conclusions: DHA for AK treatment was associated with a good clinical response and high compliance. DHA was well tolerated, with no associated phototoxicity or photoallergy, allowing treatment during summer, even in a sunny climate.

P072

Downregulation of DNA repair by an antimycotic drug leads to a phenotype resembling Xeroderma pigmentosum and increased skin cancer

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Prophylactic protection of patients with severe immunosuppression such as bone marrow transplantation individuals is of vital importance to shield the patient from opportunistic fungal infections.

It has been reported, that an antimycotic drug used for this purpose may increase the risk of subsequent development of skin tumors.
Here we report, that treatment with this antimycotic drug leads to suppression of the DNA repair mechanism nucleotide excision repair (NER) in cell based assay (such as Unscheduled DNA Synthesis and Comet assay) and increases DNA damage. Individuals with a genetic defect in NER develop Xeroderma pigmentosum, a rare skin disease, clinically characterized by pigmentary changes and a 1000 fold increased skin cancer risk. Interestingly, patients treated with this antimycotic drug develop a clinical phenotype that closely resembles that of XP. Patients develop pigmentary changes as well as skin tumors such as squamous cell carcinomas, thus reporting the link between this antimycotic drug and symptoms of NER.
Importantly the repair suppressive effect was transient since removal of antimycotic drug eventually lead to normalization of all repair associated parameters. Further studies are necessary to elucidate the full effect of antimycotic prophylaxes (i.e. time to tumor development). These findings indicate that it is important to screen patients with severe immunosuppression and antimycotic prophylaxes for early indications of an XP like phenotype as potential early warning for the development of skin tumors.

P073

Increased prevalence of metabolic syndrome in patients with acne inversa

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Acne inversa (AI; also designated as Hidradenitis suppurativa) is a destructive and scarring inflammatory skin disease with a prevalence of 1–4% affecting the axillary, inguinal and perianal skin areas. Chronic inflammation in defined diseases, such as rheumatoid arthritis and psoriasis, is associated with the metabolic syndrome and its consequences including arteriosclerosis, coronary heart disease, myocardial infraction, and stroke. So far, the association of AI with systemic metabolic alterations was largely unexplored. Thereby we carried out a hospital-based case–control study in 80 AI patients and 100 age- and sex-matched control participants. We found that the prevalence of central obesity (odds ratio 5.88), hypertriglyceridemia (odds ratio 2.24), hypo-HDL-cholesterolemia (odds ratio 4.56), and hyperglycemia (odds ratio 4.09) in AI patients was significantly higher than in controls. In consequence, the metabolic syndrome was more common in the AI patients compared to controls (40.0% vs 13.0%; odds ratio 4.46; $P < 0.001$). Even 20% of all AI patients compared to only 5% of all controls were positive for more than three of the metabolic syndrome-defining criteria. Interestingly, there was no correlation between the disease severity, duration of illness, or age of AI patients and the levels of respective parameters or the number of fulfilled criteria defining the metabolic syndrome in AI patients. Rather, the metabolic syndrome was observed in a disproportionately high percentage of very young AI patients (odds ratios for the prevalence of metabolic syndrome were >20 for Ü 34-year-olds AI). Surprisingly, there was no difference for any of the criteria for the metabolic syndrome between patients that never had surgical intervention and patients that already had therapeutic surgical interventions. This study shows for the first time a high prevalence of the metabolic syndrome and all of its criteria in AI patients. This is of particular importance since this also affects very young AI patients.

P074

Depression is a frequent comorbidity in acne inversa

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Acne inversa (AI; also designated as Hidradenitis suppurativa) is a common chronic inflammatory disease that leads chronically to painful eruptions and extensive scarring in predominantly intimate areas. It has a profound impact on the patients' quality of life. We hypothesized a higher level of depression in AI patients and thereby by means of a validated questionnaire [The Hospital Anxiety and Depression Scale (HADS-D)] we assessed depression in 44 AI patients and in 41 age-, gender-, and BMI-matched controls. In addition, we measured the blood levels of c-reactive protein (CRP) in order to evaluate the relationship between potential depression and inflammation. In our study we determined for the first time that patients suffering from AI have a higher depression score than matched controls. Importantly, 27% of AI patients are affected by depression compared to 0% of the age-, gender-, and BMI-matched controls. Surprisingly, there was no correlation between the duration of illness or age of AI patients and depression score. However, the severity of cutaneous alterations and the CRP blood levels correlated positively with the degree of depression. We concluded from our study that patients suffering from AI have a higher risk of developing depression. Furthermore, we underscore the need for physicians to implement attention on a possible development of depression when treating patients for this disease.

P075

Low dose oral isotretinoin for seborrhoea control – a randomized clinical, laboratorial and quality of life study to assess efficacy and safety. Preliminary results

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Background/Objective: Seborrhoea is a common and chronic skin disorder that affects 30% of general population characterized by shiny appearance of body sites with high number of sebaceous glands. In 3% of the cases, this condition can be associated to seborrheic dermatitis. The role of Malassezia yeasts in seborrhea and seborrheic dermatitis remains unclear. There has been reported a variety of topical and systemic antifungal agents for treatment of seborrhea and seborrheic dermatitis because their ability to reduce Malassezia yeasts. However, in severe cases some studies revealed that the systemic antifungal therapy presented no better improve than topical one. The oral isotretinoin comprises one of the final therapies for reduce sebum secretion and there are few reports about possible benefit of it in seborrhea and seborrheic dermatitis control. The purposes of this pioneer study are to perform the clinical, sebum secretion rate on the scalp and the face and the quality of life assessments of the patients classified as having seborrhea, and to perform the microbiology and molecular identification of Malassezia species isolated from scalp of these patients prior the treatment with low dose oral isotretinoin and therefore, comparing to combination topical therapy.
Material and methods: This study has been conducted after approval by Institutional Review Board and following Good Clinical Practices. Study subjects selected by inclusion and exclusion criteria signed the consent form. It is a prospective, comparative and randomized study comprising parallel groups. Final study population will be compounded by 60 healthy subjects (women and men), aged from 18 to 40, presenting seborrhea on scalp and face. Patients have been evaluated according to the following items: oily skin (face/scalp), erythema (face/scalp), desquamation (face/scalp). For each parameter a four-point score has been used – 0, absent; 1, mild; 2, moderate; 3, severe. To be included patients are required to have a sum equal to at least 4 but with oily scalp score not smaller than 2. The treatment and control groups include 30 subjects each. Subjects from control group have been treated by ciclopirox olamine shampoo three times a week cleanser to wash out the face twice a day. After standard laboratorial evaluation (lipid profile, liver function and pregnancy exclusion) subjects from treatment group take oral isotretinoin, 10 mg a day, every other day, with rigorous control on childbearing women risk. Treatment duration comprises 6 months, with safety and efficacy evaluation. Efficacy has been evaluated by clinical parameters (patient and investigator opinions), sebum measure rate (SebumeterTM, Derma Unit SSC3, Courage-Khazaka, Germany) and quality of life assessment.
Results: By now, study population comprised 14 subjects (71.4% female; 28.6% male; mean age 31 7 years; eight in control group and six in treatment group) who presented moderate to severe seborrhea. Seven of them have finished 6 months therapy. For these patients, the clinical assessment and the subject opinion were similar at the baseline and after 6 months therapy. No significant difference was noticed between groups regarding the sebum secretion rate at the baseline and after treatment. The treatment group subjects presented marginally better quality of life than the control ones ($P = 0.068$).
Conclusions: At this time, preliminary data are presented, however, final conclusions may not be performed and therefore the final results can be different.

P076

Degranulation of basophilic granulocytes from patients with extrinsic atopic dermatitis is slowed down during treatment with omalizumab

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Omalizumab, an anti-human-IgE antibody, was shown to be effective in allergic asthma, in IgE-mediated rhinoconjunctivitis and in IgE- and not IgE-mediated forms of chronic uricaria (CU). In atopic dermatitis (AD), its benefit is less clear.
Six adult patients with exacerbated AD/hyper-IgE-syndrome (>2000 kU/l total serum IgE, medium to high SCORAD, >8 weeks no systemic immunosuppressive therapy) were recruited and treated twice with 150 mg Omalizumab s.c. at week 4 and 8 in addition to standard topical therapy plus cetirizine (EudraCT# 2010-022864-12). EDTA-blood was obtained at timepoints 0, 4, 8 and 12 weeks. Cells were stimulated with (i) an anti-FceRI antibody, (ii) fMLP, (iii) ionomycin and PMA. Using the extended Flow CAST® kit (Bühlmann, Switzerland) upregulation of CD63 and CD203c on the basophil surface was quantified in a FACS-CANTO flow cytometer 15 and 45 min after stimulation. Basophils were identified by CCR3-expression and by granularity in the side scatter, gated and analysed using the DIVA-software.
Four weeks after the first dose of Omalizumab was given, the portion of both, $CD63^{pos}$ as well as $CD203c^{pos}$ basophils inducible by fMLP was significantly reduced as compared to levels before treatment ($P < 0.05$). However, no influence on anti-FceRI or PMA/ionomycin induced CD63/CD203s expression was found at this time point. Four weeks after the second injection, ionomycin/PMA induced degranulation and activation of basophils 15 min after stimulation of cells *in vitro* was reduced significantly as well. SCORAD dropped down in all six patients.
These data suggest that (i) Omalizumab mediates part of its beneficial clinical effects by interference with the cellular degranulation machinery responsible for the release of histamin-containig granules and that (ii) at least some of AD-patients refractory to standard therapy may profit from Omalizumab.

P077

Localized Bullous pemphigoid restricted to the anal region. Clearing and maintenance by topical clobetasol and pimecrolimus treatment

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Bullous pemphigoid (BP) is an autoimmune blistering disease of the skin arising in patients older than 60 years. BP usually presents as a generalized form, however, isolated forms may occur. An isolated affliction of the anal region has not been reported before. We report a 74-year-old male patient who presented with erythema, bullae and skin erosions of the anal region that had lasted for 6 months and who had been misdiagnosed with hemorrhoids. A skin biopsy revealed subepidermal blistering with a marked inflammatory infiltrate with an edematous base at the bottom of the blister exhibiting eosinophils, leukocytes, lymphocytes and neutrophils consitent with BP. Immunofluorescence microscopy revealed at the dermo-epidermal junction linear deposits of C3 and IgG. Indirect immunofluorescence exhibited circulating anti-basal membrane IgG antibodies. Immunoblot analysis showed auto-antibodies against BP 180 and BP 230 proteins.
We treated this patient by topical clobetasol treatment for about 4 weeks followed by long term treatment with pimecrolimus cream applied about five times weekly for 2 years. BP has not relapsed ever since. To summarize, this is the first report of localized BP of the anal region. We conclude that besides clobetasol, topical maintenance treatment with pimecrolimus cream is sufficient to prevent a relapse in BP restricted to the anal region.

P078

Anti-IL-23p19 (MK-3222): effects on the hallmarks of inflammation in psoriasis

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Psoriasis is a common chronic skin disease with high morbidity. IL-23 is a key driver of inflammation in psoriasis primarily released by keratinocytes, dendritic cells and macrophages. Using the anti-IL23p19 antibody MK-3222, the present study assessed the safety, tolerability, pharmacokinetics and clinical efficacy by selective targeting of IL-23 (via the p19 subunit) in psoriasis. Patients either received placebo or MK-3222 at doses of 3 or 10 mg/kg, respectively, at week 0, 4 and 8. To further investigate the impact of MK-3222 on the cellular components of the psoriatic skin infiltrate, we subjected 88 biopsies from a subset of patients ($n = 22$) included in the study at our dermatological department to a detailed immunohistochemical analysis. Biopsies were obtained before dosing and after therapy with MK-3222/placebo at week 12.
Clinically, patients receiving MK-3222 showed dose-related improvement of disease severity, as compared to placebo treatment, up to 100%.
Psoriatic lesions, when compared to non-lesional skin, were marked by increased epidermal thickness and strong expression of Keratin 16 and Ki-67 in keratinocytes, indicating extensive epidermal hyperproliferation. The amount of CD31+ vessels in the dermis was increased in lesional skin when compared to non-lesional skin.

The dermal inflammatory infiltrate in psoriatic lesions predominantly consisted of CD3+, CD4+ and CD68+ leukocytes and, to a lesser extent, of BDCA-2+ plasmacytoid dendritic cells, CD11c+ myeloid dendritic cells, CD15+ neutrophils and CD8+ cytotoxic T cells.
After treatment with MK-3222 the observed epidermal alterations in lesional skin resolved and were comparable to non-lesional skin. Remarkably, a significant reduction of CD3+ T-cells, plasmacytoid dendritic cells, myeloid dendritic cells, neutrophils and macrophages/histiocytes in the inflammatory infiltrate was seen. However, the number of CD8+ T-cells as well as Langerin+ Langerhans cells was not affected by MK-3222 treatment.
Importantly, psoriatic skin contained a significant amount of p19+ target cells in the epidermal and dermal compartment, which were completely abolished after treatment with MK-3222.
Our data shows that administration of MK-3222 in psoriatic patients induces a marked reduction of cutaneous inflammation.
The clinical and immunohistological improvement observed strongly suggests that targeting of IL-23 by anti-p19 treatment controls downstream inflammatory pathways important for disease development.

P079

The role of Thymosin-beta-4 in the pathogenesis of cold induced urticaria

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Background: Thymosin-beta-4 (Tb4), a highly conserved ubiquitary 5 kDa peptide, was recently described as a mast cells activating protein in-vitro. Additionally, ac-SDKP, the N-terminal sequence of Tb4, that can be generated by a single cleavage step has been identified as a potent activator of HMC-1 and C57 mast cell lines.
Objective: We want to test whether Tb4 might play a role in the pathogenesis of cold urticaria patients.
Method: 70 human sera from cold urticaria patients and 30 human sera from healthy subjects were assesed for Tb4 levels by ELISA. For measuring MC activation by Tb4 in-vitro we used the human MC-line LAD-2 and freshly isolated human foreskin MCs and determinded the release of ß-hexosaminidase. To determine the in-vivo mast cell activation we measured the increase of ear thickness after injection of Tb4 in genetically MC-deficient and wild type mice.
Results: We found increased Tb4 levels in the sera of cold urticaria patients (270 $\pm$ 102 ng/ml) as compared to healthy subjects (153 $\pm$ 19.12 ng/ml). Preliminary *in-vitro* experiments did not show an activation of MCs after incubation with increasing concentrations of Tb4 or ac-SDKP. In contrast, intracutaneous injection of Tb4 into the ears of wildtype, but not MC-deficiant mice, resulted in a statistically significant increase in ear-thickness.
Conclusion: These data indicate that Tb4 can activate MCs in-vivo. Elevated levels of Tb4 indicate that Tb4 or its metabolite ac-SDKP may be pathogenetically relevant in cold urticaria but further studies are needed to assess their role and relevance in this condition.

Dermato-Endocrinology

P080

A novel role for relaxin-2 in the pathogenesis of primary varicosis

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Varicose veins affect up to 40% of men and up to 51% of women. The pathophysiology of primary varicosis is poorly understood. Theories ranging from incompetence of the venous valves to structural changes in the vein wall have been proposed.
We analyzed the functional state of the intramural smooth muscle cells (n = 14 pairs matched for age and gender) and the expression of relaxin-2 and its receptors RXFP1 and RXFP2 in samples of varicose and healthy great saphenous veins (GSV) (n = 21 healthy GSV; n = 46 varicose GSV). Relaxin-2 and RXFP1 contents were determined in tissue samples (n = 9 samples per group). Pharmacological analyses were performed in a perfusion chamber.
Morphometric determination of the nuclear size of the smooth muscle compartment yielded no significant difference in varicose GSV in comparison with the healthy controls. Relaxin-2 and its receptors were expressed in the muscular layer, endothelial cells and in blood vessels contained in the vein wall. Immunohistochemical expression of relaxin-2, RXFP1 and RXFP2 was significantly decreased in varicose GSV. Relaxin-2 and RXFP1 measured by ELISA and Western Blot were decreased in varicose GSV (relaxin-2 ELISA healthy vs varicose GSV: 12.490.66 pg/mg vs 9.123.39 pg/mg of total protein; P = 0.01; Student's T-test). Contractions of vein samples induced by cholinergic or adrenergic stimulation were antagonized by relaxin-2.
We report that relaxin-2 and its receptors RXFP1 and RXFP2 are expressed in GSV and that their expression is significantly decreased in varicose GSV. Further, we were able to demonstrate a functional pharmacological relaxin-2 system in varicose GSV. Our results suggest a novel role for relaxin-2 in the pathogenesis of primary varicosis, rendering relaxin-2 a novel possible pharmacological agent for the treatment of this widely prevailing venous disease.

P081

Human Merkel cells: derived from epidermal or neural progenitors? An immunohistochemical approach

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Merkel cells (MCs), neuroendocrine cells of the skin, may originate from an epidermal or a neural crest lineage. In mice, MCs are derived from epidermal stem cells during ontogenetic development, and these stem cells also ensure MC homeostasis in adult mice. Given that MC distributions in human and mouse skin are significantly different, it remains to be clarified whether the MC population in human adult skin is replenished from an epidermal progenitor pool as well. The route of differentiation from stem cells to specialized cells often includes transitory stages. During these stages, stem cell marker proteins and proteins typically produced by the respective differentiated cell may be expressed in parallel, as has been demonstrated for astrocytes and melanocytes. It is thus likely that a similar transition state exists during MC differentiation. If human MCs are derived from the epidermal lineage, one would expect a co-expression of epidermal markers and MC markers in transitory cells, whereas the 'neural crest origin hypothesis' would predict co-expression of neural crest markers and MC markers. To test these hypotheses, we investigated human scalp skin by double immunofluorescence analyses, combining either epidermal stem cell markers or neural crest markers with the MC marker cytokeratin 20 (CK20). CK15, Leucine-rich repeats and immunoglobulin-like domains protein 1 (Lrig1), and p63 were employed as epidermal stem cell markers. To label neural crest-derived stem cells or their immediate progeny, we used anti-Sox10 antibodies. Lrig1 and CK20 displayed an overlapping expression in 20% of CK20-positive cells in the interfollicular epidermis and 7% of CK20-positive cells in hair follicles. An overlap between CK20 and either CK15 or p63 was only observed in 3% or less of CK20-positive cells. Moreover, none of the Sox10-immunopositive cells exhibited CK20 staining. Interestingly, whereas MCs were frequently in contact with one or more cells expressing Lrig1, CK15 or p63, we only very rarely observed Sox10-positive cells adjacent to MCs. These data suggest that human MCs in scalp skin may rather originate from epidermal than from neural crest progenitors, and that the Lrig1/CK20-coexpressing cells may represent transient MC precursors. The transcription factor NFI-A and the transcriptional cofactor BTG2, which we found to be expressed in a subset of CK20-positive cells, are likely to regulate MC differentiation from stem cells. In ongoing experiments, we analyze the potential co-expression of CK20 with additional proteins typically present either in epidermal stem cells or in neural crest-derived stem cells.

P082

Key hormones of the hypothalamic-pituitary-thyroid (HPT) axis regulate mitochondrial biology in human hair follicles *in situ*

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Elements of the hypothalamic-pituitary-thyroid axis (HPT) are transcribed in human skin and we already reported that thyrotropin-releasing hormone (TRH) and thyrotropin (TSH) are important regulators of the mitochondrial biology of human skin epithelium. Assuming that hair follicle cycling and hair shaft elongation are highly energy-demanding processes, we asked whether and how TRH, TSH and the thyroid hormones triiodothyronine (T3) and thyroxine (T4), impact on the mitochondrial biology of human scalp hair follicles. This was examined by treating microdissected human scalp hair follicles with T3 (100 pM), T4 (100 nM), TRH (30 nM) or TSH (10 mU/ml) for 24 h in serum-free organ culture.
By qRT-PCR T3 and T4 significantly increased gene expression of the mitochondrial-encoded subunit 1 of cytochrome c oxidase (MTCO1) and of the mitochondrial transcription factor A (TFAM), by quantitative immunohistomorphometry we found an increased protein expression of TFAM by all the tested hormones whereas MTCO1 protein expression was increased by TSH, T4 and T3.
Additionally, TRH, TSH, T4 and T3 stimulated mitochondrial energy metabolism as demonstrated by an increased activity of the respiratory chain complex I and complex IV in homogenized HFs. This was confirmed by increased heat production (as measured by chip-calorimetry) after T3 treatment. Furthermore, intrafollicular mitochondrial biogenesis was up-regulated by all the tested HPT axis hormones. This was demonstrated by a significant increased number of ultrastructurally detectable perinuclear mitochondria and by an increased protein expression of porin, the voltage-dependent-anion-channel (VDAC1), expressed in the outer membrane of the mitochondria. Those results correlate with the increased expression of TFAM, the final effector of the mitochondrial DNA duplication.
Since heightened mitochondrial activity could also exert undesirable effects (e.g. increased oxidative stress due to an increased ROS production), outer root sheath keratinocytes were tested for ROS production after hormonal treatment, both at baseline level and after H_2O_2 stimulation. The treatment with the HPT-axis hormones did not affect the basic oxidation state of these hair follicle keratinocytes, although T3 and T4 tended to prevent increased ROS production after H_2O_2 stimulation. Moreover, by qRT-PCR, catalase transcription was enhanced by TRH, TSH, T4 and T3 in hair follicle keratinocytes, whereas SOD2 transcription was increased just by TSH and T4 treatment.
Thus, our results show that mitochondrial function, energy metabolism and redox state of human hair follicles are subject to profound (neuro-)endocrine regulation by all key hormones of the HPT axis (TRH, TSH, and T3), two of which are generated in loco. This invites novel (neuro-)endocrine approaches to the treatment of human hair growth.

P083

Withdrawn

P084 (O21)

Characterization of the oxytocin system in human skin

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The neuropeptide hormone oxytocin (OXT) mediates a wide spectrum of tissue-specific actions, ranging from cell growth, cell differentiation, sodium excretion to stress responses, reproduction and complex social behaviours. Recently, OXT expression has been detected in keratinocytes, but expression of its receptor and function are still unexplored in human skin. Here, we show that both, OXT and its receptor, are expressed in primary human skin cells, including keratinocytes, melanocytes, dermal fibroblasts and human dermal microvascular endothelial cells. OXT induces dose-dependent calcium-fluxes in dermal fibroblasts and keratinocytes, indicating that the OXT receptor (OXTR) is functionally expressed in both celltypes. We also show that OXT decreases proliferation of dermal fibroblasts and keratinocytes in a dose-dependent manner *in vitro*. In order to further investigate OXT-mediated functions in the skin, we performed OXTR-knockdown experiments. OXTR-knockdown in dermal fibroblasts lead to elevated levels of reactive oxygen species. In keratinocytes an increased release of pro-inflammatory cytokines, such as IL-6, RANTES and CXCL10, was observed. Atopic dermatitis, a multifactorial, inflammatory skin disease, is characterized, among others, by epidermal hyperproliferation and an increased susceptibility to oxidative stress. We detected a reduced expression of the OXT system in lesional and non-lesional atopic skin suggesting a clinical relevance in skin homeostasis.

P085

IGF-1 activates phosphoinositide-3-kinase (PI3K)/Akt/FoxO1 pathway in SZ95 Sebocytes *in vitro*

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A new hypothesis of acne pathogenesis suggests that acne-inducing factors exert their action by reducing nuclear transcription factor FoxO1 levels via activation of the phosphoinositide-3-kinase (PI3K)/Akt/FoxO1 pathway and consequently activate androgen receptor and key genes of cell proliferation, lipid biosynthesis and inflammatory cytokines. A low glycemic load diet has been shown to improve acne and reduce free insulin-like growth factor-1 (IGF-1) activity. IGF-1 is known to activate pAKT and induce sebocyte differentiation. The aim of this study was to investigate the effect of IGF-1 in the regulation of AKT and FoxO1 activation and cellular functions of SZ95 sebocytes.
SZ95 sebocytes were stimulated with different concentrations of IGF-1 in a time-dependent manner and expression levels of pAKT, AKT, pFoxO1 and FoXO1 were analyzed by Western Blot. Proliferation

of SZ95 sebocytes was measured by [3H] thymidine incorporation assay at different time points from 24 h to 6 days after IGF-1 incubation and differentiation by semiquantitative analysis of lipid droplet accumulation using Oil Red staining.
The western blotting results revealed early up-regulation of p-Akt and a delayed up-regulation of p-FoxO1 60–90 min after stimulation with IGF-1 1 M. [3H] thymidine incorporation assay revealed significant suppression of SZ95 proliferation upon incubation with IGF-1 0.1 M after 72 h and 6 days and 0.01 M after 6 days ($P < 0.01$). In addition, lipid production analyzed by Oil Red staining showed a time-dependent significant increase in SZ95 differentiation.
In this study, we show for the first time that IGF-1 activates both pAKT and pFoxO1 in SZ95 sebocytes and that FoxO1 might be involved in the regulation of IGF-1-induced increased differentiation and decreased proliferation of SZ95 sebocytes *in vitro*. These data support the potential role of FoxO1 as a key molecule in the pathogenesis of acne.

P086

Prolactin and prolactin receptor are expressed in human corporal skin, and substance P, interferon gamma and tumour necrosis factor alpha represent novel regulators of prolactin expression in human skin and hair follicles

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It remains unclear whether corporal human skin expresses prolactin (PRL) and PRL receptor (PRLR) and how their expression is locally regulated. Prolactin and PRLR protein expression were confirmed by immunohistochemistry in the corporal skin of three different female subjects. Interestingly, PRL gene expression, measured by real time PCR, increased during serum-free skin organ culture, which corresponded with an observed increase in PRL protein expression. This provides firm evidence for intracutaneous PRL production. In contrast, PRLR expression decreased in organ culture, at both the gene and protein level. Neither dopamine, the key regulator of pituitary PRL secretion, nor corticotropin-releasing hormone, had any effect on the expression of PRL and/or PRLR in organ-cultured human scalp hair follicles (HFs) and skin at the gene and protein level. In contrast, Substance P and tumour necrosis factor alpha decreased PRL protein expression in skin and HFs respectively, while interferon gamma increased PRL immunoreactivity (IR) in HFs. However, there was no evidence of PRL secretion into the culture media. This study confirms that both PRL and PRLR are expressed in female human corporal skin, and that they are regulated during organ culture. Substance P, tumour necrosis factor alpha, and interferon gamma are important regulators of intracutaneous PRL and PRLR expression, at least *in vitro*. Given the emerging importance of PRL in inflammatory dermatoses, including psoriasis, targeting these regulators of cutaneous PRL expression may represent a novel treatment strategy.

P087

The alpha 7 nicotinic acetylcholine receptor – an emerging target counterregulating inflammatory responses of epidermal keratinocytes

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Epidermal keratinocytes are constantly exposed to ultraviolet (UVB) light which induces a plethora of proinflammatory cytokines including tumor necrosis factor-alpha (TNF-alpha). Recently, we found that the serotonin receptor (5-HT-R) antagonist tropisetron, an approved antiemetic, not only suppressed collagen synthesis in dermal fibroblasts but also elicited antifibrotic effects in the bleomycin mouse model of scleroderma (Stegemann et al., Arthritis & Rheum, in press). Interestingly, this modulatory effect of tropisetron in fibroblasts was mediated via alpha 7 nicotinic acetylcholine receptors (alpha7nAchR) but not 5-HT-Rs. Since bleomycin-induced fibrosis is considered as an inflammation-mediated model of experimental fibrosis we wondered if tropisetron via activation of the same receptor has also anti-inflammatory effects in normal human keratinocytes (NHK). Tropisetron at 1 g/ml to 1 ng/ml doses significantly suppressed UVB- and TNF-alpha-induced mRNA expression of interleukin (IL)-6, IL-8 and cyclooxygenase-2. This effect of tropisetron on UVB- and TNF-alpha-mediated IL-6 and IL-8 expression was confirmed at protein level. Mechanistically, tropisetron attenuated TNF-alpha- and UVB-mediated nuclear translocation of p65/NF-B but neither affected p38-signaling nor IBα-degradation. Next we performed expression analysis of the putative receptors for tropisetron in NHK. Interestingly, both 5-HT3-R and 5-HT4-R were undetectable in NHK at RNA and protein level while expression of alpha7nAchR was present. Alpha-bungarotoxin, a specific alpha7nAChR antagonist neutralized whereas the full alpha7nAchR agonist AR-R17779 mimicked the effect of tropisetron on UVB-mediated cytokine IL-6 and IL-8 expression in NHK. Taken together, our findings strongly suggest that tropisetron via alpha7nAChR elicits counterregulatory effects on both UVB- and TNF-alpha-mediated inflammatory responses in NHK. Moreover, alpha7nAChR may be an emerging target for the future design of anti-inflammatory drugs in dermatology.

P088 (O14)

Reduction of UVA-induced oxidative stress via regulation of catalase - a novel photoprotective mechanism of alpha-melanocyte-stimulating hormone in cutaneous biology

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Irradiation with ultraviolet A (UVA) is a key pathogenetic factor during cutaneous photoaging. UVA exposure typically induces reactive oxygen species (ROS) and hydrogen peroxide which in turn upregulate expression of matrix metalloproteinases (MMPs). We hypothesized that alpha-melanocyte-stimulating hormone (alpha-MSH), which previously was shown to reduce UVB-induced DNA damage, may also exhibit indirect anti-oxidative effects in human dermal fibroblasts (HDFs) exposed to UVA. In addition, we speculated that photoaging would be increased in red haired pale skin individuals carrying loss of function mutations of the melanocortin-1 receptor (MC1R) which binds alpha-MSH with high affinity. HDFs pretreated with physiological amounts of alpha-MSH for 24 h exhibited significantly reduced intracellular amounts of ROS after UVA exposure. The type and subcellular localization of the detected oxidative stress in response to UVA treatment was found to be mainly hydrogen peroxide within the cytoplasm as determined by various organelle-specific fluoroprobes, incubation with cell-permeable superoxide dismutase, and exogenous catalase. Importantly, a functional MC1R was essential for the suppressive effect of alpha-MSH on UVA-induced oxidative stress in HDFs. Agouti signalling protein, a natural MC1R antagonist, blocked the protective effect of alpha-MSH. In accordance with this, HDFs carrying loss of function mutations of MC1R (R151C or R160W) displayed increased basal levels of intracellular hydrogen peroxide and failed to respond to alpha-MSH. Importantly, the modulatory effect of alpha-MSH on UVA-induced oxidative stress was paralleled with reduced expression of both MMP1 and MMP3, key enzymes of dermal photoaging. To finally identify the molecular mechanism by which alpha-MSH exerts its UVA-photoprotective effect, we performed gene knock down of catalase, an enzyme which degrades hydrogen peroxide. siRNA of catalase but not control siRNA completely abrogated the suppressive effect of alpha-MSH on UVA-mediated accumulation of hydrogen peroxide in HDF. In support of this, alpha-MSH increased enzyme activity but not mRNA and protein expression of catalase in a time- and dose-dependent manner in these cells. In summary, these findings add a novel twist to our current understanding how the cutaneous UVA response is regulated by the alpha-MSH/MC1R system.

P089

KdPT: a novel small molecule protective against impaired wound healing in diabetes?

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Skin ulcers are a major complication in diabetic patients. An ideal future agent useful for effective therapy of such ulcers is expected to be inexpensive, non-degradable, antimicrobial, anti-inflammatory and stimulatory on keratinocyte proliferation and migration. Moreover, excessive accumulation of reactive oxygen species (ROS) is a well described phenomenon in diabetic tissues demanding for an agent with anti-oxidative and/or cytoprotective properties. The melanocortin tripeptide derivative KdPT may be such a putative compound since our previous research has provided compelling evidence for a pleiotropic anti-inflammatory, cytoprotective, and anti-oxidative action of this peptide. Therefore, we assessed the modulatory impact of KdPT on high glucose-induced effects on normal human keratinocytes (NHK) in culture. High glucose adversely affected a panel of functional *in vitro* parameters, i. e. cell proliferation, metabolic activity, viability and two-dimensional migration. However, no signs of classical apoptosis or autophagy were observed after high glucose. Using atomic force microscopy high glucose was also found to profoundly alter the biomechanical properties of stressed NHK, i. e. diameter and elasticity. Importantly, glucotoxicity was paralleled by significant induction of intracellular ROS and endoplasmic reticulum stress. KdPT significantly attenuated high glucose-induced oxidative stress and antagonised the toxic effects of glucose on cell viability, metabolic activity and migration. Preliminary results on skin organ cultures further indicate that KdPT maintains these salutary effects also in a more pathophysiologically relevant scenario. In summary, our findings highlight a novel modulatory effect of KdPT which may be exploited for the future therapy of diabetic skin ulcers.

P090

Nox4 – an emerging target for the future treatment of scleroderma

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The pathogenesis of systemic sclerosis (SSc) is still incompletely understood and effective therapies are urgently needed. Key pathogenetic events are profibrotic cytokines like transforming growth factor-β1 (TGF-β1) leading to increased extracellular matrix synthesis and induction of myofibroblasts. Of note, there is also accumulating evidence that abnormal expression and activity of distinct NADPH oxidase (Nox) isoforms are crucially involved in some fibrotic diseases, e. g. in lung fibrosis (Hecker et al. Nature Med 2009). Here, we analyzed the expression pattern of Nox enzyme isoforms in neonatal and adult human dermal fibroblasts (HDF) as well as in HDF from patients with SSc. We also investigated the regulation and function of the detected Nox isoform in the context of collagen synthesis and myofibroblast activation. Normal and diseased HDF expressed mRNA transcripts for Nox4 as well as its associated adaptor proteins p22phox and Poldip2. In contrast, Nox1, Nox2/gp91phox (the principal Nox isoform present in macrophages), Nox3, Duox2, p47phox, P67phox and p40phox were undetectable in these cells. Importantly, stimulation of normal HDF with TGF-β1 dramatically upregulated Nox4 expression at mRNA and protein level in a time-dependent manner as determined by real-time RT-PCR and Western immunoblotting. Immunofluoresence analysis revealed that TGF-β1-mediated induction of Nox4 protein localizes to the cytoplasm as well as to the perinuclear space of the cells. Pharmacological inhibition of Nox enzyme activity by diphenyleneiodonium not only suppressed TGF-β1-mediated expression of collagen type I but also induction of both α-smooth muscle actin (α-SMA) and fibronectin 1, established myofibroblast markers. To finally test if agents suppressing experimentally induced fibrosis likewise reduce NOX4 expression, we pretreated HDF with various agents including α-melanocyte stimulating hormone (α-MSH). Pretreatment but not cotreatment with α-MSH in fact suppressed TGF-β1-mediated Nox4,α-SMA and fibronectin 1 expression in a dose-dependent fashion supporting a modulatory role of this neuropeptide on fibroblast activation and ECM production. In summary, our results describe a novel and unexpected function of a specific NADPH isoform, Nox4, in fibroblast biology of the skin. Our findings are also encouraging to further dissect the functional role of this NADPH isoform in experimentally induced fibrosis models of the skin in order to tailor more effective therapies against fibrotic skin diseases.

P091

Extracellular calcium and 1,25 dihydroxyvitamin D3 significantly increase SZ95 sebocyte plating efficiency and cell amount and modulate lipid synthesis *in vitro*

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Background: Calcium (Ca^{2+}) and 1, 25-dihydroxyvitamin D3 (calcitriol) are well-known promoters of several epithelial cell functions; however their effects on sebocytes are not clearly elucidated.
Methods: To evaluate extracellular Ca^{2+} and calcitriol effects on SZ95 sebocyte morphology, plating efficiency, proliferation, apoptosis and lipid synthesis *in vitro* following incubation in a serum-free Sebomed® medium supplemented with different concentrations of extracellular Ca^{2+} (0.05–1.4 mM) and with and without addition of calcitriol (10^{-9} or 10^{-7} M) at 24 and 72 h in culture.
Results: At 72 h, SZ95 sebocytes maintained at low Ca^{2+} (0.05 mM) had rounded cell morphology; remained as individual cells or formed few loose colonies that poorly expanded and tended to detach from culture plates. In contrast, SZ95 sebocytes maintained at high Ca^{2+} (1.4 mM) were polygonal in shape, readily expanded and formed large compact colonies firmly adherent to culture plates. Extracellular Ca^{2+} concentration significantly increased SZ95 sebocyte numbers in a dose-dependent manner, being highest at Ca^{2+} concentration of 1.4 mM and lowest at 0.05 mM at 24 (increased plating efficiency) and 72 h (increased proliferation) ($P < 0.01$ and $P < 0.001$ respectively). However, SZ95 sebocyte lipid synthesis decreased with higher Ca2+ concentrations ($P < 0.01$) at 24 and 72 h pointing out to an inverse relationship with SZ95 proliferation. Low extracellular Ca^{2+} resulted in significant SZ95 cell apoptosis as assessed by caspase 3/7 activity (1.9- to 2.1-fold increase, $P < 0.001$) and further confirmed ultrastructurally by presence of features of cell death in the form of prominent lipid vacuoles and increased number of lysosomes (autophagic vacuoles; both were clearly evident at 72 h. Calcitriol showed a significant time- and dose-dependent increase of SZ95 cell numbers likely by enhancing the extracellular Ca^{2+} effect ($P < 0.001$). On the other hand, only at 72 h calcitriol was associated with a significant decrease of lipogenesis mostly detected at a concentration of 10^{-7} M and with higher Ca^{2+} concentration (1.4 mM), further

confirming the reciprocal relationship between SZ95 cell proliferation and lipid synthesis ($P < 0.001$). Calcitriol at both concentrations had no effect on SZ95 cell amount in the presence of low Ca^{2+} level (0.05 mM) at both 24 and 72 h ($P < 0.05$).
Conclusions: Extracellular Ca^{2+} and calcitriol regulate sebocyte morphology and increase both plating efficiency and cell growth but decrease lipid synthesis *in vitro*. These biological events may have a major impact on the treatment of sebaceous gland-associated diseases.

P092

Differential expression of nuclear vitamin D receptor (VDR) cofactors contributes to the resistance of melanoma cell lines against the antiproliferative effects of 1,25-dihydroxyvitamin D3 (1,25(OH)2D3)

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The active vitamin D metabolite 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) and analogs represent promising new compounds for treatment of various malignancies, including melanoma. 1,25(OH)2D3 exerts antiproliferative and differentiation-inducing effects on tumor cells via its corresponding intranuclear vitamin D receptor (VDR). However, we demonstrated previously that about half of melanoma cell lines are resistant to the antiproliferative effects of 1,25(OH)2D3. So far, the molecular mechanisms which are responsible for this resistance are unclear. First results point at decreased VDR-mediated transcription of target genes. We now have analyzed the expression of nuclear cofactors that contribute to VDR-mediated gene transcription. Expression of VDR, RXRs, CYP24A1, nuclear coactivators of VDR (steroid receptor co-activators (SRCs), vitamin D receptor interacting proteins (DRIPs), nuclear coactivators (NCoAs)), and corepressors of VDR (SMRT and NCoR) was investigated in vitamin D sensitive (MeWo, SkMel5) and resistant (MelJuso, SkMel25, IGR) melanoma cells treated with and without 1,25(OH)2D3 using real time PCR and western analysis. There was no difference in basal VDR mRNA and protein expression comparing untreated 1,25(OH)2D3-responsive and -resistant cell lines. However, 1,25(OH)2D3-responsive melanoma cell lines showed after 1,25 (OH)2D3-treatment a stronger increase in VDR (up to 2.4-fold) and CYP24A1 (up to 7000-fold) expression compared with 1,25(OH)2D3-resistant cell lines (up to 1.4-fold and up to 70-fold, respectively). The expression profile of RXRα before and after treatment with 1,25(OH)2D3 was comparable in 1,25(OH)2D3-responsive and -resistant cell lines both at the protein and mRNA level. Interestingly, we observed differences in the expression of VDR cofactors that may be of relevance for differences in transcription of VDR target genes. For example, expression of DRIP100 was stronger induced after 1,25(OH)2D3-treatment in 1,25(OH)2D3-responsive as compared to -resistant cell lines. Expression of NCoA3 was partially stronger repressed after treatment with 1,25(OH)2D3 in 1,25(OH) 2D3-responsive compared to -resistant cell lines. In summary, we here report differential expression of nuclear VDR cofactors in 1,25(OH)2D3-responsive compared to -resistant cell lines that may be at least in part be responsible for the resistance of melanoma cell lines against the antiproliferative effects of 1,25(OH)2D3.

P093

Genetic variants (SNPs) of genes involved in skin pigmentation are associated with 25(OH)D serum concentration

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Background: Vitamin D deficiency is common in the Caucasian population. Low serum 25(OH)D concentrations are associated with an increased incidence and an unfavourable outcome of many diseases, such as various types of cancer, infectious, cardio-vascular, and autoimmune diseases. Individual factors that predispose for a person's vitamin D status, such as skin type, have been identified. However, there are limited data on genetic determinants of serum 25(OH)D concentration.
Patients and methods: We tested the hypothesis that variants of genes involved in skin pigmentation are predictive of serum 25(OH)D levels. Serum 25(OH)D levels and single nucleotide polymorphisms (SNPs, $n = 960$) of genes ($n = 29$) involved in skin pigmentation were retrospectively analyzed in a cohort of participants of the LURIC study ($n = 2970$).
Results: 47 SNPs of genes involved in skin pigmentation were associated with significantly ($P < 0.05$) lower or higher serum 25(OH)D levels as compared to the total cohort (Median: 15.5 ng/ml). Interestingly, two SNPs were associated highly significant ($P < 0.0005$) with low serum 25(OH)D levels. The following SNPs were associated with the lowest serum 25(OH)D concentrations (Median): rs16949906 (DCT), 5.1 ng/ml, $P = 0.009$; rs1408797 (TYRP1), 7.2 ng/ml, $P = 0.023$; rs12530875 (PRKAR2B), 8.2 ng/ml, $P = 0.039$; rs16929263 (TYRP1), 8.3 ng/ml, $P = 0.009$; rs17342340 (TYRP1), 8.3 ng/ml, $P = 0.011$; rs17407577 (FGF2), 8.8 ng/ml, $P = 0.015$; rs8034368 (OCA2), 8.8 ng/ml, $P = 0.026$; rs16950402 (OCA2), 8.8 ng/ml, $P = 0.027$. The following SNPs were associated with the highest serum 25(OH)D concentrations (Median): rs6123895 (EDN3), 21.8 ng/ml, $P = 0.034$; rs389673 (CTNNB1), 19.25 ng/ml, $P = 0.024$; rs10747595 (ATF1), 18.95 ng/ml, $P = 0.025$.
Conclusions: We have identified variants of genes involved in skin pigmentation that are predictive of serum 25(OH)D levels in the Caucasian population.

P094

Glutamate metabolism is a target in the aging process: a study in human hair follicles

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Aging is a growing issue in the western societies as associated diseases impose great challenges with respect to costs and management. Graying is a hallmark of the aging process and may be investigated as a model-phenomenon to detect hitherto un-investigated and safe targets to halt the aging process. We previously reported differential gene expression in pigmented, gray and white human scalp skin hair follicles from identical donors. These included non-melanocyte related genes alongside the expected changes in melanogenesis associated genes, among them genes associated with energy metabolisms that pointed at alterations in glutamate utilization (glutaminase, guanidine monophosphate synthetase, glutamate decarboxylase 1). These results were confirmed by PCR and immunohistochemistry. Subsequently, we performed functional analysis employing microsurgically isolated human hair follicles submitted to organ culture. We supplemented cultured hair follicles with different concentrations of glutamine and found that this treatment altered the *in vitro* hair follicle aging process and melanocyte biology. In detail, suboptimal glutamine concentrations in the culture medium failed to support healthy growth (elongation, Ki67 expression, TUNEL assay, routine-histochemistry dystrophy-assessment) and induced *in vitro* graying together with altered expression of migration, differentiation and senescence markers in cultured human hair follicles. Taken together, we conclude that analysis of graying hair follicles is a useful model to investigate the aging process and that altered energy metabolism is involved in it.

P095 (O27)

Allergic inflammation in the skin modifies the central stress response in a neuropeptide and neurotrophin dependent manner

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Allergic inflammation is associated with an attenuated response of the hypothalamus pituitary adrenal axis (HPA) e.g. in female patients with atopic dermatitis resulting in reduced cortisol levels after acute stress exposure. At the same time increased production of cytokines such as interleukin (IL) 4, IL 5 or IL 10 was frequently reported in response to stress in patients with allergic inflammatory disease together with a worsening of disease parameters. But whether enhanced inflammation was cause or result of altered stress perception is not clarified. From animal experiments we learned that substance P (SP) can block HPA activation at the level of the hypothalamus and that peripheral allergic inflammation generates high levels of neuroplastic nerve growth factor (NGF) which may potently modify the response to stress in allergic inflammation. Here we employ a combined model of inflammatory and perceived stress in syngeneic C57BL/6 mice to investigate this interaction. We found that the presence of an atopic dermatitis-like allergic dermatitis (AlD) does not increase the total number of activated cFos + neurons in the hypothalamus. However, a solitary increase of cFos+ neurons was found in the dorsomedial nucleus at bregma level 1.58 (DM), activation of which can block HPA activation by stress. By contrast, exposure to 24 h of noise-stress did increase the total number of cFos+ neurons in the hypothalamus. Intriguingly, the presence of allergic inflammation in the skin of AlD mice reduced this stress-induced increase in the number of cFos + neurons alongside with increased SP endcoding hypothalamic PPT1-mRNA levels in the hypothalamus and reduced stress-induced peripheral corticosteroid levels. At the same time, stressed AlD mice altered anxiety-like behavior. Moreover, the increase in cFos+ neurons in the DM of AlD mice was blocked by additional treatment with SP-neutralizing NK-1 blocker and the PPT1 increase in stressed AlD mice was blocked by peripheral injection with NGF-neutralizing antibodies. Taken together, NGF generated by peripheral allergic inflammation may alter HPA function and behavior via modified SP expression and regulate stress-sensitivity in individuals affected by atopic disease.

P096

Chronically perceived stress during exam affects healthy young women: consequences for stress-mediator release and TH17 immunity

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Stress has been identified as a major risk factor for hampered tissue regeneration after inflammatory injury in many experimental and clinical settings. Actual data from the mouse model hints at a close interaction between chronic stress exposure and a shift of the immune balance towards adaptive humoral immunity. Exam stress is a paradigm frequently employed to dissect the human neuroendocrine-immune responses to stress. Here we report that the subjective perception of anxiety and nervous mood as measured by the state and trait anxiety index (STAI) and the multidimensional mood questionnaire (MDMQ) were the most prominent psychometric changes characterising chronic stress perception in the exam group throughout a 12 week examination preparation and execution period in healthy young women undergoing the final medical exam at the Humboldt-University in Berlin, Germany. These results corresponded well with reduced morning serum cortisol levels 12 weeks prior to exam (exam preparation) and during exam execution when compared to expression levels in female medical students participating in a regular semester. At the same time, serum level of the neurotrophin brain derived neurotrophic factor (BDNF) was significantly increased. On the immunological side only the summary score for cytokines conducting the TH17 response differed significantly form controls as early as 12 weeks prior to the exam. These results link classical stress axis modification with altered neurotrophin expression with TH17 driven inflammation possibly explaining the deleterious effects of long term stress exposure on TH17 driven inflammatory diseases such as psoriasis.

P097

Further investigation of the pathogenetic role of subtilisin kexin isoenzyme-1 in human melanoma

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Subtilisin-kexin isozyme-1 (SKI-1) is one of the most recently identified members of the prohormone convertase (PC) family. PCs typically process precursor hormones but can also activate other substrates, e.g. transcription factors, growth factors, growth factor receptors and matrix metalloproteinases. While there is increasing evidence that some PCs are involved in tumorigenesis and metastasis of keratinocyte-derived tumors their role in melanoma pathobiology is still poorly investigated. We recently found that SKI-1 is constitutively expressed at mRNA and protein level in normal human melanocytes and eight human melanoma cell lines. Decanoyl (dec)-RRLL-chloromethylketone (CMK), a cell-permeable pharmacological SKI-1 inhibitor, led to a dose-dependent inhibition of the metabolic activity and proliferation of normal melanocytes and melanoma cells. Interestingly, the growth inhibitory effect of CMK *in vitro* was significantly more pronounced in melanoma cells. Further investigation revealed that SKI-1 is secreted into the culture media of melanoma cells. Employing an *in vitro* SKI-1 enzyme assay, we found that SKI-1 activity in cell culture supernatants (but not in cell lysates) is significantly higher in most melanoma cell lines than in normal melanocytes. In accordance with this, SKI-1 enzyme activity was higher in tissue homogenates of melanoma metastases than in normal skin. To identify the molecular mechanism by which SKI-1 inhibition leads to reduced cell viability of melanoma cells *in vitro* we performed various read-outs for programed cell death. Treatment of melanoma cells with CMK resulted in a clear-cut induction of apoptosis as shown by cell death detection ELISA, Annexin-V staining and processing of poly-adenosine diphosphate-ribose polymerase 1/2. To finally identify the putative SKI-1 substrate responsible for CMK-mediated apoptosis of melanoma cells we investigated the processing and subcellular distribution of ATF6. This transcription is activated by SKI-1 and interestingly, ATF6 was recently reported to exert an essential role in survival of HepG2 liver cells in absence of endoplasmic reticulum stress. In fact, treatment of melanoma cells with CMK reduced the protein levels of processed ATF6α, i. e. AFT6α-p50, and further led to nuclear exclusion of the processed isoform of ATF6. Taken together, these findings show an essential role for SKI-1 in melanoma cell growth and survival and highlight several novel molecular targets for the future therapy of this tumor.

P098

Prohormone convertase PC5/6 – an emerging player in melanoma biology?

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Prohormone convertases (PCs) are Ca^{2+}-dependent serine proteases that were initially characterized as enzymes processing precursor hormones. However, there is increasing evidence that distinct PC members can also process and activate a number of non-hormone substrates including growth factors, growth factor receptors, adhesion molecules, extracellular matrix degrading enzymes, and transcription factors. Accordingly, we recently found that two members of the PC family, paired basic amino acid-cleaving enzyme-4 and subtilisin kexin isoenzyme-1, exert unexpected functions in melanoma biology by regulating tumor cell growth and survival. Here, we investigated the putative role of PC5/6 in melanoma cells. Real-time RT-PCR analysis of normal human melanocytes (NHM) and eight human melanoma cell lines derived from different stages of disease progression in fact revealed that PC5/6 mRNA expression is significantly elevated in the majority of tested melanoma cell lines compared to NHM. This result could be confirmed at protein level by Western immunoblotting in which PC5/6 protein expression was confined to melanoma cells. PC5/6 protein expression could be further localized within the cytoplasm of melanoma cells as shown by double immunofluorescence analysis. Next, we wondered how expression of PC5/6 is regulated in response to growth factors, cytokines and cellular stressors known to affect cell proliferation and survival. Using *in silico* promoter analysis we first identified consensus sequences for AP-1, NF-B, CREB and ATF6 in the PC5/6 promoter suggesting regulation of PC5/6 gene expression by binding of activated transcription factors to these sites. Treatment of NHM with selected stimuli activating the above transcription factors demonstrated that tunicamycin, an inducer of endoplasmic reticulum stress and activator of ATF6, indeed induced PC5/6 mRNA and protein expression in a time-dependent fashion. We are currently underway to define the precise role of PC5/6 in melanoma cells by siRNA-mediated gene knock-down. This treatment resulted in marked suppression of constitutive PC5/6 expression in one representative melanoma cell line, WM9. The consequences of siRNA-mediated gene knock-down of PC5/6 will next be determined by measuring metabolic activity, proliferation, apoptosis and invasiveness *in vitro*.

P099

Prospective investigation of 25(OH)D3 serum concentration following UVB narrow band phototherapy in patients with psoriasis and atopic dermatitis

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Vitamin D deficiency represents a major health issue. It is a worldwide endemic and associated with a broad variety of severe diseases. The skin is a key tissue for the human body's vitamin D endocrine system. It represents a target tissue for biologically active vitamin D metabolites. Approximately 90% of the human body's requirements of vitamin D have to be synthesised in the skin by the action of UVB-radiation. However, the factors that influence the cutaneous synthesis of vitamin D are still not well understood. In our present prospective study we investigated the effect of UVB narrow band (UVBnb, 311 nm) and PUVA phototherapy on 25(OH)D3 serum concentration in patients with psoriasis, atopic dermatitis and a few cases with other dermatoses ($n = 57$). We found that 2 weeks of UVBnb treatment resulted in an increase of 25(OH)D3 serum concentration from 11.4 to 20.5 ng/ml ($P < 0.001$), while in contrast PUVA-treatment didnt significantly alter vitamin D status. These findings question the hypothesis of an relevant vitamin D metabolizing effect of UVA. Psoriasis patients showed a trend for a stronger increase in 25 (OH)D3 serum levels following UVBnb compared to patients with atopic dermatitis. Patients with relatively low baseline serum 25(OH)D3 concentrations had a stronger increase in 25(OH)D3 concentrations compared to patients with relatively high 25(OH)D serum concentrations. In general patients with skin types (Fitzpatrick) I and II (median 14.3 ng/ml) had a higher baseline of 25(OH)D3 serum concentration compared to patients with skin types III (median 11.2 ng/ml) or IV-V (median 12.3 ng/ml), although these differences were statistically not significant ($P = 0.106$). Baseline and increase in 25(OH)D3 serum concentrations following UVB phototherapy were correlated with presence of genetic variants (SNPs of VDR, GC) that influence vitamin D status. Moreover we analysed the impact of other individual factors such as body mass index, age and gender on 25(OH)D3 serum concentrations following UV-phototherapy. When we investigated the effect of phototherapy on blood pressure and a variety of laboratory parameters such as CRP, HbA1c, LDL, HDL, triglycerides and cholesterol no consistent significant effects were found. In summary our study characterises the impact of various relevant factors on 25(OH) D3 serum concentration following UV phototherapy (UVBnb and PUVA) in patients with psoriasis and atopic dermatitis.

P100

Alterations of sebum composition triggers acne vulgaris

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For a long time hyperseborrhea has been considered as the major etiopathogenetic factor for the development of acne. However, sebum quantity *per se* cannot be the only responsible factor, as demonstrated by the success of treatment with agents with no primary effect on sebum excretion rate, such as antibiotics, topical retinoids, azelaic acid and benzoyl peroxide. In addition, changes in sebaceous gland activity not only correlates with changes in sebogenesis but also with alterations in sebum fatty acid composition. However, it is only currently that first the oxidant/antioxidant ratio of the skin surface lipids and newly alterations of lipid composition have been taken into consideration in the aetiopathogenesis of acne and other skin diseases. Lower essential fatty acid levels were found in wax esters in twins with acne rather than in twins with no acne. Moreover, low levels of linoleic acid (LA) have been observed in skin surface lipids of acne patients, topical LA application reduces microcomedones and increased steroid 5α-reductase activity exhibits an inhibitory effect on LA. Sebum contains LA, an essential fatty acid that cannot be synthesised *in vivo* and therefore must be obtained from the diet. It has recently been hypothesised that low glycemic load diet may influence sebum production based on the beneficial endocrine effects of its components. On the other hand, unsaturated fatty acids in sebum alter the calcium dynamics in epidermal keratinocytes and induce abnormal follicular keratinization and comedone formation. Increased IL-1α levels are a hallmark of comedogenesis and, while *P. acnes* is unable to induce IL-1α expression in the pilosebaceous unit, oleic acid causes keratinocyte toxicity and increased IL-1α mRNA levels. In addition, lipids at the skin surface, mostly secreted onto the surface from the sebaceous glands, are part of the innate immunity of the skin and contribute to the antimicrobial skin barrier. The ω9 fatty acids oleic acid and the sebaceous gland specific palmitic acid and sapienic acid are very effective against *Staphylococcus aureus*, whereas dysfunction of the upstream lipidogenic enzymes stearoyl-CoA desaturase and fatty acid desaturase 2 leads to skin infection and inflammation. Interestingly, peroxidation of squalene, another sebaceous gland-specific lipid, and other lipids leads to comedogenesis. All these data strongly indicate a central role of sebaceous lipid quality and not quantity on the development of acne and other skin diseases.

P101

Treatment algorithm of hidradenitis suppurativa/acne inversa

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Hidradenitis suppurativa/acne inversa (HS) is a chronic, recurrent, follicular skin disease usually presenting after puberty and been potentially scaring. It manifests with painful, deeply localized, inflammatory skin lesions that occur in apocrine gland-rich areas of the skin, most commonly in the axillae and the inguinal and anogenital regions (Dessauer definition). The recognized trigger factors include smoking and obesity. The treatment of HS is often disappointing, and has a significant negative impact on patients' quality of life. Regarding stressfulness and reduction of life quality HS ranks on the top among all dermatological diseases. There are no approved drugs for HS treatment. According to empirical analyses of treatment measures only topical 1% clindamycin solution, the oral systemic combination of clindamycin and rifampicin, the hormonal antiandrogen combination of ethinyl estradiol and cyproterone acetate, the biologics infliximab and adalimumab, and surgery reached an evidence level 2 and a grade B recommendation. To accomplish a grade-relevant treatment, the HS experts group of the German Dermatological Society (DDG) proposed the following treatment algorithm: Hurley's grade I: Topical 1% clindamycin solution treatment followed by systemic clindamycin 300 mg 2–3×/day (or minocycline 2 × 50 mg/day) and rifampicin 300 mg 2×/day p.o. for 4–12 weeks, with clindamycin 300–600 mg 2–3×/day administered i.v. during the first 5 days of treatment. For women with signs of hyperandrogenism/hyperandrogenemia oral antiandrogen hormonal therapy with ethinylestradiol/cyproterone acetate (up to 100 mg/day) should be administered. Hurley's grade II: Like in grade I followed by limited excision of recurrent lesions (alternatively ablation with the CO_2 laser). Hurley's grade III: Like in grade I followed either by wide excision or by infliximab infusion 5 mg/kg body weight once or twice (after 1 week) or adalimumab (160 mg s.c. and 80 mg 1 week later if required) to reduce the lesional and perilesional inflammation, followed by the wide excision of the involved area.

P102

Detection of melanocortin receptors in human eccrine sweat gland epithelia *in situ* and *in vitro*

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Basic research on sweat glands largely remains a terra incognita in investigative dermatology. However, it is well known that the function of eccrine sweat glands is orchestrated by a plethora of neuroendocrine mediators and classical hormones, some of which are even produced within the skin. Interestingly, it is known for years that the melanocortin 5 receptor (MC5R) is expressed in various exocrine glands in rodents where it may regulate the functional state of these organs. Nevertheless, with regard to eccrine sweat glands the role of melanocortin receptors and their ligands is largely unexplored. We demonstrate that the melanocortin-1 receptor (MC1R), which binds melanocyte-stimulating hormones as well as adrenocorticotropin, is markedly expressed in secretory cells of the glandular portion of eccrine sweat glands in adult human skin. Moreover, cytoplasmic MC1R immunoreactivity was observed in the outer cell layer of the sweat duct and acrosyringium but not in myoepithelial cells. Next, the human eccrine sweat gland-derived immortalized NCL-SG3 cell line was used as an *in vitro* model. Expression analysis of all known MCR subtypes by semi-quantitative RT-PCR with established primer sets confirmed the presence of MC1R in NCL-SG3 cells. MC1R immunoreactivity was confirmed by double immunofluorescence analysis employing an antibody directed against the extracellular portion (aa 2–18) of the human MC1R. Interestingly, NCL-SG3 cells also expressed MC3R transcripts but not those for MC2R, MC4R and MC5R. In contrast to other cell types of human skin, NCL-SG3 cells did neither express POMC mRNA nor protein indicating that these cells do not produce melanocortin peptides in an autocrine or paracrine fashion. In summary, our preliminary findings highlight the existence of distinct melanocortin receptor subtypes in human eccrine sweat gland epithelia. Based on these results further studies can be done to precisely determine whether and how MCR ligands as well as their antagonists affect the functional state, e. g. differentiation and proliferation, apoptosis or ion transport, of eccrine sweat epithelia *in vitro* and *in vivo*.

P103

Endocannabinoids regulate connective tissue and mucosal type mast cell activation and maturation via cannabinoid receptor-1 *in situ*

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Background: Mast cells (MCs) play a crucial role in the development of many chronic inflammatory and allergic disorders. In such diseases, excessive MC activity and number are observed in the tissues. To investigate the role of MCs in the pathogenesis and clinical phenotype of such diseases, it is important to understand how healthy human tissues that are rich in MCs (such as skin or bronchial mucosa) avoid excessive MCs activity and their total number under physiological circumstances.

Objective: Since endocannabinoids are increasingly recognized as neuroendocrine regulators of MC biology, here we investigated how cannabinoid receptor-1 (CB1) mediated-signaling affects human MCs i.e. connective tissue type MCs (CTMCs) and mucosal type MCs (MMCs) *in situ*.

Methods: This was investigated in the MCs-rich connective tissue sheath (CTS) of organ-cultured human scalp hair follicles (HFs) for CTMCs, and in the mucosa of organ cultured human nasal polyps for MMCs, by quantitative (immuno)histomorphometry, ultstrastructural and qPCR techniques, using CB1 agonists, CB1 antagonist, or CB1 gene knock-down.

Results: Kit+ MCs within the CTS of human HFs express functional CB1 receptors, whose pharmacological blockade (using specific CB1 antagonist, AM 251) or CB1 gene specific silencing significantly stimulated both MCs degranulation and maturation from resident progenitor cells *in situ* (i.e. enhanced the number of tryptase+, FcεRIα or chymase+ CTS-MCs). However, CB1 blockade did not affect MCs proliferation. The effect of CB1 blockade on MCs was, at least in part, stem cell factor (SCF)-dependent. The prototypic endocannabinoid, anandamide (AEA), and the CB1-selective agonist, arachidonyl-2-chloroethylamide (ACEA) counteracted the MCs-activating effects of classical MCs secretagogues, including substance P and compound 48/80. Similar results were obtained within the nasal mucosa of human nasal polyps. Kit+ MMCs express functional CB1 *in situ*. Blockade of CB1-signaling enhanced MMCs degranulation and increased their total number without affecting their proliferation *in situ*. MMCs maturation was induced at least in part via up-regulating SCF production.

Both AEA and ACEA effectively counteracted secretagogue-triggered excessive MMCs degranulation *in situ*.
Conclusion: Using human HF organ-culture and nasal polyp organ-culture as clinically relevant model systems for investigating MC biology *in situ*, we show that normal CTMCs and MMCs are tightly controlled by the endocannabinoid system. This limits excessive MCs activation and maturation from resident progenitors via 'tonic' CB1 stimulation by locally synthesized endocannabinoids. The excessive MCs number and activation in allergic and other chronic inflammatory diseases may partially arise from resident intracutaneous MC progenitors, e.g. due to insufficient CB1-stimulation. Therefore, CB1-stimulation is a promising novel strategy for the future management of allergy and MCs-dependent diseases.

P104

Human epithelial stem cells need 'tonic' cannabinoid receptor-1 mediated signaling

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Background: Cannabinoid (CB) receptor-mediated signaling has recently become accepted as an important modulator of epithelial cell biology and pathology (e.g., epidermal growth and differentiation). We have previously shown that microdissected human hair follicles (HF)s express CB1 and human HF growth is controlled by CB1 signaling. However, the role of CB1 signaling in epithelial stem cell (SC) biology remains to be explored.
Objective: To understand the role of CB1 mediated signaling on epithelial SCs in human skin, we focused on cytokeratin (CK)15+ SCs within the HFs as well as the epidermis.
Methods: To investigate the role of CB1 signaling on human HF SCs, we performed human HF organ culture. Furthermore, we used isolated human epithelial SC populations to investigate that on interfollicular epithelial SCs.
Results: Here we show that CK15+ SCs and their progenitors express functional CB1 *in situ*. The CB1 specific agonist, arachidonoly-2 chloroethanolamine (ACEA) increased CK15-promoter-GFP activity *in situ* and *in vitro*. The number of CK15+ progenitors, and their proliferation, was increased by ACEA treatment. CB1 gene-knockdown in organ-cultured human HFs decreased the number of CK15+ progenitors. While their proliferation was not significantly reduced, CB1 gene-knockdown significantly increased their apoptosis. Similar phenomena was observed in human interfollicular epidermal SCs suggesting that the proliferation-stimulatory effects of CB1 agonists are a general feature of adult human skin epithelial SCs. In stark contrast, ACEA inhibited the proliferation of human HF outer root sheath (ORS)-keratinocytes and increased their apoptosis. The MEK inhibitor, but not PI3K or p38 MAPK inhibitor abrogated the ACEA-induced increased number of CK15+ progenitors *in situ*. In addition, ACEA increased Erk expression in CB1+ isolated human bulge cells, suggesting that CB1-stimulation is mediated, at least in part, by the MEK/Erk pathway. Since CB1-stimulation promotes reepithelialization of organ-cultured, wounded human skin, while bulge CB1 expression is reduced in scarring alopecia (lichen planopilaris), CB1 signaling is clinically relevant.
Conclusions: Taken together, these data suggest that CB1-mediated signaling is required for the maintenance of epithelial progenitor/SCs but not their differentiated progeny. Therefore, endogenous CB1-agonists, such as the endocannabinoids anandamide and 2-AG, may have a major impact on human epithelial progenitor biology and this may indicate that the endocannabinoid system is a novel future target in the management for the diseases characterized by the damage of epithelial SCs, including scarring alopecia and chronic wounds.

P105

Wnt signalling is a key regulator of human skin ageing

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WNT represents a large morphogenic family of secreted lipid-modified glycoproteins that control multiple developmental processes during embryogenesis such as cell fate specification, progenitor cell proliferation and the control of asymmetric cell division. In addition, they have been associated in adult tissues and organs with tissue maintenance, remodelling and cancer progression. In previous microarray studies, we showed that one of the conserved pathways which exhibited decreased regulation with age in human skin of 24 female and male European Caucasian donors was the WNT signalling. The list of overlapping genes revealed four genes of this pathway, which were downregulated with age, namely AXIN2, FZD7, WIF1 and CPZ. Further investigation of the WNT signalling via real time PCR and immunohistochemistry confirmed our findings. At RNA level, WIF1 expression was downregulated in female and male aged skin (36%; $P < 0.01$ and 69%; $P < 0.01$, respectively), whereas AXIN2 expression was only downregulated in female aged skin (29%; $P < 0.01$). At protein level the expression of FZD7 and WIF1 was negative in the skin of elderly subjects. On the other hand, the young group showed a higher expression of both proteins ($P = 0.0019$ and $P = 0.013$, respectively) in the basal cell layer of the epidermis only. No significant gender differences were observed. This is the first report linking WNT signalling pathway with human skin ageing. Understanding the mechanisms of ageing in humans as well as their gender classification can form the basis for comprehensive, knowledge-based prevention of age-related diseases and extension of healthy lifespan through the development of preventive and therapeutic agents and procedures.

P106

Novel pattern of sebaceous differentiation and lipogenesis induced by the -9 fatty acid palmitic acid

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Stimulation of sebocytes with the short and long chain polyunsaturated fatty acids linoleic acid (LA) and arachidonic acid promotes their differentiation and lipogenesis *in vitro*. On the other hand, the effect of -9 fatty acids in sebocyte physiology is not yet fully elucidated. Sebocytes are known to utilize the -9 fatty acid palmitic acid (PA) for the production of palmitoleic and sapienic acid and for the production of sebum wax esters, while PA upregulates the expression of antimicrobial peptides in SZ95 sebocytes *in vitro*. PA is the most abundant saturated free fatty acid in the blood stream known to promote cell death or dysfunction to various cell types: It induces apoptosis of cardiomyocytes and pancreatic -cells, promotes necroptosis of endothelial cells, and is the cause for mitochondrial reactive oxygen species formation in hepatocytes. In our experimental setting the role of PA in sebaceous differentiation and lipogenesis was investigated using stimulation with LA as control. SZ95 sebocytes were seeded in confluent and subconfluent conditions and were treated with 5×10^{-5} M PA or LA. The concentrations did not appear to have any cytotoxic effect on the cells according to LDH levels in the supernatants. Neutral lipid content correlated to amount of total protein was measured with Nile Red/BCA assay after 24, 48 and 72 h. LA induced a stronger neutral lipid accumulation pro g of total protein in comparison to PA after 24 h. On the other hand, the neutral lipid levels after stimulation with the two fatty acids after 48 h were equal. IL-6 and IL-8 ELISA revealed that LA induced higher SZ95 IL-6 expression in comparison to PA for all time points, a finding that was confirmed by immunocytochemistry. In contrast, PA induced higher IL-8 expression than LA, especially after 72 h. From the differentiation markers tested in order to determine the pattern of fatty acid-induced sebaceous differentiation, the mucin-cancer associated antigen (MCA) was already downregulated after 24 h for LA. Expression of human-milk-fat globulin 1 (HMFG-1) was induced by LA and significantly upregulated by PA for all three timepoints. In addition, the epithelial sialomucin MAM-6 was upregulated by PA after 48 h in confluent conditions. From the aforementioned data, we provide evidence for a novel pattern of -9 fatty acid-induced sebaceous differentiation and lipogenesis, which offers new perspectives in understanding sebaceous gland physiology and pathology.

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The Th1/Th17 cytokine-milieu favours NALP1 inflammasome activity and IL-1 release in psoriasis

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IL-1 is a key player in cutaneous inflammation and promotes the development of the Th17 micro-milieu important in the pathogenesis of psoriasis. AIM2 and NALP3 inflammasome components have been detected in the psoriatic epidermis, however, little is known about the functional contribution of inflammasomes for epidermal IL-1 release in psoriasis. Here, we discover the NALP1 inflammasome functionally active in human epidermal keratinocytes and showed that NALP1 associated IL-1-converting enzymes caspase-1 and caspase-5 are up-regulated in lesional psoriatic skin. We further identified the Th1 cytokine IFN as a key regulator of both caspase-1 and caspase-5 in epidermal keratinocytes, whereas the Th17 cytokines TNF and IL-17A synergized to induce the inflammasome substrate pro-IL-1. The psoriatic epidermis is exposed to a mixed Th1/Th17 cytokine environment. In combination, IFN and IL-17A amplified the expression NALP1 inflammasome components, whereas the AIM2 complex was down-regulated in epidermal keratinocytes. Together, Th1/Th17 cytokines control the inflammasome activity in human skin keratinocytes. Data suggest that the psoriatic micro-milieu shifts inflammasome activity towards NALP1 that is important for IL-1 release by the psoriatic epidermis.

P108

Conversion of skin fibroblasts of patients with Alzheimer's disease into induced pluripotent stem cells

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Alzheimer's disease (AD) is a neurodegenerative disorder mostly linked to increasing age. It is characterized by a loss of neuronal cells in hippocampus area controlling cognitive balance, memory and behavior. At present there are no markers to diagnose AD at early stage and no therapy to abort its progression. Induced pluripotent stem cells (iPS) comparable to human embryonic cells are a current major issue in biomedicine. They offer an enormous potential for disease research, drug screening, toxicology and regenerative medicine. However, little is known about the nature and sequence of molecular events accompanying nuclear reprogramming. In order to further investigate skin-system organs associations, we performed cell infection with retroviruses expressing the four transcriptional factors OCT4, SOX2, KLF4 and C-MYC and we generated iPS cells from dermal fibroblasts obtained from a healthy neonate (BJ, HFF1, purchased from ATCC) and from an 81-year-old patient diagnosed to suffer from AD (NFH-46). The iPS colonies, which formed 4 weeks after infection, exhibited the essential characteristics of embryonic stem cells, such as alkaline phosphatase activity, expression of cell surface markers and pluripotency-associated genes.

P109

Single nucleotide polymorphisms (SNPs) in genes involved in the vitamin D endocrine system are associated with risk for cutaneous basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs)

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Vitamin D deficiency represents a major health issue. It is a worldwide endemic and associated with a broad variety of severe diseases, including various types of cancer. Recently, a protective effect of vitamin D compounds against the carcinogenesis of cutaneous basal cell carcinomas (BCCs) and the photocarcinogenesis of squamous cell carcinomas (SCCs) was shown, via regulation of hedgehog- and p53 signaling pathways, respectively. We have now tested the hypothesis that single nucleotide polymorphisms (SNPs) in genes involved in the vitamin D endocrine system are associated with risk for BCCs and/or SCCs using multivariate analysis. DNA was extracted from paraffin embedded BCCs ($n = 245$), SCCs ($n = 277$) or from blood of healthy controls ($n = 376$). SNPs were analyzed in genes encoding for CYP27B1 (rs4646536), CYP24A1 (rs927650, rs2762939), VDBP (rs1155563, rs7041), and VDR (rs757343, rs731236, rs2107301, rs7975232). Several SNPs were significantly ($P < 0.05$) associated with risk for BCCs and/or SCCs. Interstingly, impact on risk for BCCs and SCCs was different for most SNPs analyzed, and several SNPs predispose for the progression of actinic keratoses to SCCs. In summary, we here demonstrate that several SNPs in genes involved in the vitamin D endocrine system differentially contribute to the pathogenesis of BCCs and SCCs.

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Induced pluripotent stem cell-derived neurons from a sporadic Alzheimer's disease donor as a model for investigating disease mechanisms

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Alzheimer's disease (AD) is a complex, irreversible neurodegenerative disorder mostly linked to increasing age. At present there are no reliable markers to diagnose AD at an early stage and no therapy to abort its progression. Thus, currents efforts are directed at the investigation of the underlying disease mechanisms. To attain this goal, induced pluripotent stem (iPS) cells offer unprecedented opportunities, as they allow the generation of patient-derived neuronal cells in a dish. In this study, we developed and characterized iPS cells from dermal fibroblasts of an 82-year-old female patient affected by sporadic AD at late stage. The AD-iPS colonies exhibited the essential characteristics of embryonic stem cells. We then obtained AD-iPS-derived neuronal cells which expressed p-tau and GSK-3β, a physiological kinase of p-tau. In a proof of principle treatment of neurons derived from AD-iPS with an inhibitor of β-secretase, we could confirm the down-regulation of p-tau in AD neurons from one AD-iPS line. Furthermore, transcriptome analysis of AD neurons revealed a significant change in expression of a number of genes involved in AD pathogenesis, these include, PSEN1, BACE1, and BACE2, and the dysregulation of oxidative stress and apoptosis-related biological processes. Our study demonstrates how an iPS-based model system could represent a suitable tool for studying the underlying molecular basis of sporadic Alzheimer's disease and may eventually depict novel therapeutic avenues for this debilitating neuronal disorder.

P111

Impact of arachidonic acid and staurosporine on apoptosis of SZ95 sebocytes

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The etiology of several sebaceous-associated diseases is not fully clarified. Understanding of their molecular background is, therefore, strongly required to develop and administer appropriate treatments. The establishment of human immortalized sebocytes (SZ95) has facilitated the investigation of the molecular pathophysiology of sebaceous gland and sebocyte function, meeting a need since the significance of sebaceous gland research is steadily increasing.

The study was undertaken to investigate the influence of the proinflammatory free fatty acid arachidonic acid and of the cytostatic staurosporine on SZ95 sebocytes in a time- and dose-dependent manner.

The levels of basal apoptosis as well as cell differentiation in connection to lipogenesis in treated cells were investigated. Additionally we analyzed the expression of several possibly involved proteins in the above mentioned biological process via Western blot analysis.

An uncommon effect of staurosporine could be demonstrated at low concentrations, as it induced a significant increase ($P < 0.01$) in sebaceous lipids after 48 h of treatment. The expression of Bax, a proapoptotic protein that is absolutely required for apoptosis in many different cell types, remained unchanged. Surprisingly staurosporine also led to an increased expression of the antiapoptotic protein Bcl-2. While staurosporine caused severe apoptosis, mostly concentration-independently, high concentrations of arachidonic acid were needed to significantly elevate the apoptosis rate.

We demonstrated different effects of arachidonic acid and staurosporine on SZ95 sebocytes in dose dependent concentrations. This data may help to unveil the molecular pathogenesis of sebocytes-associated diseases and new drug discovery.

P112

Melatonin activates the transcription factor Nrf2 and phase-2 antioxidant enzymes in UV-irradiated human keratinocytes

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Melatonin (*N*-acetyl-5-methoxytryptamine) is a highly conserved naturally occurring molecule. It is present in virtually all organisms, from bacteria to plants and mammals. Melatonin is a highly lipophilic molecule that crosses cell membranes to easily reach subcellular compartments, including mitochondria. These organelles are one of the main sites of hypergeneration of reactive oxygen species, and melatonin or its metabolites exert potent radical-scavenging activities. However, the molecular mechanisms of antioxidant signaling pathways induced by melatonin are not fully elucidated, especially in human skin. Here, we studied in a dose- (0, 10, 25, 50 mJ/cm^2) and time-dependent (0, 4, 24, 48 h post-UVR) manner whether melatonin could prevent the UVR-mediated oxidative stress response (ROS formation) through nuclear erythroid 2-related factor (Nrf2) and its consecutive phase-2 and antioxidative enzymes in human keratinocytes. Our investigations revealed that UVR induces hypergeneration of reactive oxygen species directly after UV exposure (0 h post-UVR) by 12% compared to the control ($P < 0.01$) at a dose of 50 mJ/cm^2. Keratinocytes pre-incubated for 1 h with melatonin (10^{-3} M) did not show a distinct difference at 0 h post UVR exposure at a dose of 50 mJ/cm^2, however, first significant protection of melatonin was noticed after 4 h post-UVR by 21% ($P < 0.001$). Additionally, melatonin was shown to induce the translocation of Nrf2 transcription factor into the nucleus and as a result it, increased the activity of phase-2 and antioxidative enzymes such as catalase (CAT), glutathione peroxidase (GPx), heme oxygenase 1 (HO-1), NADPH quinone oxidoreductase 1 (NQO1), superoxide dismutase (SOD) and -glutamate cysteine synthetase (-GCS). Therefore, we suggest based on these data that the Nrf2 activation by melatonin might be a new crucial pathway leading to a differentially regulated antioxidative response against UVR.

P113

Melatonin counteracts UVR-induced up-regulation of HSP70 expression in human *ex vivo* skin

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Melatonin exerts its potent protective effects via the melatoninergic antioxidative system of the skin. In the skin, melatonin provides strong protection against various oxidative stress-associated apoptotic and DNA-damage conditions due mainly to the fact that melatonin and its metabolites effectively scavenge reactive oxygen species. To date, there is strong evidence for melatonin to act efficiently against ultraviolet radiation (UVR), the main inductive factor for skin damage, e.g. inflammation, oxidative and DNA damage leading to skin aging and cancer. Besides oxidative stress, one of the commonly known stress proteins induced by UVR is heat shock protein 70 (HSP70) that is highly expressed in human keratinocytes. Therefore, this investigation aimed at evaluating the potent preventing action of melatonin regarding UVR-mediated up-regulation of HSP70 in a human *ex vivo* full-thickness skin organ culture. Experiments were conducted in a dose- (0, 100, 300 mJ/cm^2) and time-dependent manner (0, 24, 48 h post-UVR). HSP70 gene expression evaluated by real-time PCR directly increased (0 h) by 2.50- and 3.30-fold ($P < 0.001$) after UVR at doses of 100 mJ/cm^2 and 300 mJ/cm^2, respectively, and was further up-regulated during prolonged culture time up to 48 h. Pre-incubation with melatonin significantly prevented HSP70 up-regulation by 43.27% (100 mJ/cm^2) and 44.85% (300 mJ/cm^2) at 48 h post-UVR. Gene expression analysis was found to be highly consistent with investigations regarding HSP70 protein in skin lysates (ELISA) as well as HSP70 protein detection *in situ* (immunofluorescence labeling). So far protective mechanisms of melatonin have been mainly attributed to its antioxidative mechanisms. Our data add a new mechanism to this scenario showing that melatonin may counteract UVR-induced up-regulation of HSP70 expression.

P114

Sulforaphane and phenylethyl isothiocyanate protect human skin from UV-induced inflammation and apoptosis

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Chronic exposure to ultraviolet radiation (UV) induces stress response and antioxidant defense mechanisms in human skin. Moreover, UV has been reported to induce inflammation and programmed cell death. The transcription factor nuclear erythroid-2 related factor 2 (Nrf2) regulates gene expression of antioxidative enzymes and may modulate anti-inflammatory and anti-apoptotic response mechanisms. In this study, we tested the isothiocyanates sulforaphane (SFN) and phenylethyl isothiocyanate (PEITC) for their ability to counteract UV-induced oxidative stress and apoptosis in an *ex vivo* human full-thickness skin tissue model. Twenty-four hours after UV irradiation at a dose of 300 mJ/cm^2, both a significant increase of sunburn cells and structural tissue alterations was observed ($P < 0.001$) with a further significant increase up to 48 h ($P < 0.001$). At this time point, a 5-fold increase of sunburn cells compared to the sham-irradiated control was found (0 mJ/cm; $P < 0.001$). Furthermore, UV irradiation induced consumption of the antioxidative enzyme catalase (CAT) directly (0 h) after UV exposure ($P < 0.001$) and up-regulation of cleaved caspase-3 positivity after 24 h with ongoing activation of this protein up to 48 h. Pre-incubation of skin with SFN or PEITC demonstrated significant protective effects of these alterations. Comparative mechanistic cell culture studies using HaCaT keratinocytes showed that SFN and PEITC increased Nrf2 activity and Nrf2-dependent gene expression such as -glutamylcysteine-synthetase (GCS), heme oxygenase 1 (HO-1) and NADPH quinone oxidoreductase 1 (NQO1). Thus, the induction of Nrf2-dependent signal transduction pathways may represent a possible mechanism by which SFN and PEITC counteract oxidative stress and apoptosis in human skin.

Dermatopathology

P115

The Alarmins Psoriasin (S100A7) and Koebnerisin (S100A15) supress the extracellular matrix production in the skin

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Background: Keloids form as a result of aberrations in the normal wound healing cascade and are frequently associated with clinical symptoms such as pruritus, contractures and pain. A genetic predisposition to keloids has been suggested; however, the complex mechanisms underlying the processes of excessive scarring are inadequately understood. Peptides of the psoriasin (S100A7)/koebnerisin (S100A15) family are expressed in keratinocytes and released during normal cutaneous wound healing. Here, we found that psoriasin and koebnerisin production is markedly decreased in keloid scar tissue compared to normal skin. We thus aimed to elucidate their role for the regulation of collagen synthesis by dermal fibroblasts.

Material and methods: The transcript abundance of COL (collagen type) 1A1, COL1A2 and COL3A1 in biopsies from keloid tissue and normal tissue was determined using real-time qPCR and confirmed by Western Blotting and immunohistochemistry. Primary fibroblasts were isolated from normal skin biopsies and were incubated with psoriasin and koebnerin peptides alone and in combination. The S100-mediated regulation of COL1A1, COL1A2, COL3A1, fibronectin, laminin, alpha SMA and TGF beta 1–3 was determined using real-time qPCR.

Results: Treatment of fibroblasts with psoriasin and koebnerisin (combined only?) downregulated levels of COL1A1, COL1A2, COL3A1, fibronectin-1, laminin beta, alpha SMA and TGF beta 1–3 in normal fibroblasts. Cell viability was not affected by either S100-protein.

Conclusion: The anti-fibrotic function of psoriasin and koebnerisin may lead to novel preventive and therapeutic strategies for fibroproliferative diseases of the skin and beyond.

P116

Inflammatory cells and Th2 cytokines in chronic spontaneous urticaria

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Leukocyte infiltration in to the skin is a feature of urticaria but the clinical significance and the mechanism(s) involved are unclear. We previously demonstrated that, compared to controls, there were increased numbers of eosinophils, neutrophils, T cells and macrophages, as well as IL-4+ and IL-5+ cells, in biopsies from lesions in chronic spontaneous urticaria (CU) suggesting that Th2 pathology may contribute to tissue swelling.

To compare biopsies from affected and unaffected skin from CU as well as normal skin for the presence of inflammatory cells and IL-25+ and IL-33+ cells.

Paired biopsy specimens from affected (4–8 h lesions) and unaffected skin were obtained from eight patients with CU. Results were compared to nine control subjects. Cryostat sections were processed for immunohistochemistry using the APAAP technique.

There were significant elevations in the numbers of intradermal MBP + eosinophils ($P = 0.009$), elastase + neutrophils ($P = 0.001$), CD3+ T cells ($P = 0.01$), CD68+ macrophages ($P = 0.01$), IL-25+ cells ($P = 0.01$) and IL-33+ cells ($P = 0.002$) when lesional skin from CU was compared to unaffected skin. The approximate fold differences in the medians were eosinophils (25), neutrophils (30), T cells (4), macrophages (0.4), IL-25 (6) and IL-33 (5). There were no significant differences in the numbers of immunopositive cells when normal controls were compared to unaffected skin from the urticaria patients.

Taken together, our results indicate that eosinophils, neutrophils and other inflammatory cells may contribute to tissues swelling in chronic urticaria through mechanisms involving Th2 cytokines.

P117 (O29)

Correlation of Merkel Cell Polyomavirus positivity with PDGFRα mutations and Survivin expression in Merkel Cell Carcinoma

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Merkel Cell Carcinoma (MCC) is a rare neuroendocrine and highly aggressive cancer of the skin. Clinically this tumor is characterized by rapid and asymptomatic growth and prevalence for UV-exposed skin. Little is known on MCC pathogenesis and oncogenesis, however more recently, a viral

involvement has been hypothesized. In previous studies the Merkel Cell Polyomavirus (MCPyV) was detected in up to 80% of the tumor specimens and although MCPyV is very ubiquitous throughout the population, the viral DNA was shown being integrated in the genome of MCC rather than in other skin tumors.
We have analyzed 50 MCC specimens for viral positivity and load. Out of these, 33 patients (68%) were MCPyV positive with viral load variable in all samples ranging from 0.006 to 318 virus DNA copies / 1 beta globin. Most of the virus positive tumors were localized on upper extremities, which are more exposed to UV light. We found viral DNA in patients with and without metastatic disease. However patients with distant metastases displayed the highest viral load. This data suggests a correlation between viral positivity and aggressive outcome of the disease.
In addition we have investigated whether the mutations in PDGFRα and expression of Survivin, previously detected in MCCs, are consequence of viral infection leading to disease progression.
Analysis of mutations in PDGFRα exons detected different mutations in exon 10, 12 and 18. In this study, 51% of MCPyV positive and 50% of MCPyV negative tumors harbored at least one mutation of the PDGFRα gene, thus suggesting that the presence of mutations was independent of viral positivity. Furthermore, PDGFRα protein expression was detected in all MCC and only 11% of the entire analyzed population showed no or low expression of this protein. Interestingly lower expression of PDGFRα was present in patients with the metastatic disease.
Survivin expression was detected in the majority of the patients (73%) with a strong to moderate expression pattern. We did not find any correlation between Survivin expression and viral positivity. Yet, it is possible that cellular localization may be correlated to disease progression where nuclear expression of Survivin is a negative prognostic factor.
Taken together, our data indicate that viral positivity correlates with poor disease outcome, but not with mutations of the PDGFRα gene or with Survivin expression suggesting that these factors independently contribute to Merkel Cell Carcinoma development.

P118

Eczema in psoriatico: further results to close the gap between palmoplantar psoriasis and palmoplantar chronic contact dermatitis. A clinical, histological and immunohistological study

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Psoriasis and chronic eczema at the palmoplantar sites show multiple overlapping clinical and histological features. Sometimes the differentiation, especially by lacking other skin changes is hardly possible. Data from our department show a large group of patients with clinical and histological features of psoriasis and contact dermatitis combined with a high frequency of positive patch tests. This constellation is mostly called 'Eczema in Psoriatico'.
The purpose of our study was to compare clinical, histological and immunohistological characteristics of these patients with typical palmoplantar psoriasis and typical palmoplantar contact dermatitis.
Two experienced dermathopathologists performed a blinded evaluation of 142 samples and a final selection of 64 patients: 33 patients with 'eczema in psoriatico', 14 patients with allergic contact dermatitis, 12 patients with psoriasis and five samples of healthy skin. Patients with marked positive patch tests results were accordingly classified. Cases with chronic irritative non allergic hand and foot manifestation were excluded from this study.
According to our experience and to the literature we have chosen histological parameters that were considered helpful in the diagnosis both of psoriasis and of contact dermatitis. H&E- stained sections were evaluated blind to clinical diagnosis. Eczema in psoriatico showed overlapping histological features with both psoriasis and contact dermatitis. The following histological findings of 'eczema in psoriatico' were shared with psoriasis: moderate to strong acanthosis, regular acanthosis, thinning of suprapapillary plates, oedema of papillary dermis. Characteristics of 'eczema in psoriatico' were seen with a similar frequency as in contact dermatitis: parakeratosis with both neutrophils and plasma exsudation, lymphocytic exocytosis in epidermis, full-thickness spongiosis and spongiotic vesicels. The pattern of rete ridges (club-shaped, V-shaped) and of tortous, dilatated capillaries in upper dermis was mixed in 'eczema in psoriatico', showing more similarities to psoriasis than to contact dermatitis. In most cases of 'eczema in psoriatico' (66.6%) there was only a partial loss or thinning of granular layer.
By immunohistochemistry 'eczema in psoriatico' showed a similar pattern to contact dermatitis and in contrast to psoriasis overexpression of CD1a- and IL 31-positiv cells and decreased expression of TLR4. However, IL23- and IL17 staining both showed similar highly positive pattern in psoriasis and 'eczema in psoriatico'. Similar expression between psoriasis and 'eczema in psoriatico' was also observed in IL8 staining. Ki67 staining was highest in psoriasis, moderate in 'eczema in psoriatico', but lowest in contact dermatitis. No statistical differences between psoriasis 'eczema in psoriatico' and contact dermatitis could be calculated with regard to CD 26 and CK 16 staining.
Our study distinguishes a group of patients with type IV sensitization and coexistence of clinical and histological features of palmoplantar psoriasis and chronic eczema. The immunohistological analysis shows that in case of 'eczema in psoriatico' there are contemporaneous immunological processes specific for both diseases.

P119

Establishment of an immunohistochemical marker panel for the diagnosis of skin graft-versus-host disease

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Graft-versus-host disease represents one of the major complications after allogeneic hematopoietic stem cell transplantation. Its predilection sites are the skin, the liver and the mucous membranes. A reliable diagnosis of GvHD is often compromised by the fact that drug reactions or viral exanthema can elicit similar clinical and histological symptoms. In fact, even experienced physicians are challenged by confirming this diagnosis. Specific immunohistochemical markers for skin GvHD are still lacking. In a nutshell, GvHD occurs when the recipient's tissue is damaged and host antigen-presenting cells (APCs) are activated by inflammatory cytokines. These cells trigger activation of donor T cells, which then attack host cells such as professional APCs and non-hematopoietic APCs. Thus, we have examined histologically and immunohistochemically skin biopsies from 19 patients with acute (aGvHD, grade 1–3 Lerner classification) and chronic GvHD of lichenoid and sclerotic type (cGvHD) after stem cell and organ transplantation with the aim of identifying a panel of immunohistochemical markers that can be used for diagnostic purposes in skin GvHD. We confirmed the diagnosis of acute GvHD by vacuolar degeneration of the basal keratinocytes, lymphoid infiltrates in epidermis and dermis as expected, as well as apoptotic cells and satellitosis of lymphocytes around apoptotic keratinocytes and correlated these with the clinical findings. In addition, we stained for Caspase 3 as a marker of apoptotic cells and found that they were predominantly present in high grade aGvHD. We did not observe alterations in dermal CD11c-, and in epidermal and dermal CD1a-positive dendritic cell numbers between control and diseased samples. In high grade aGvHD, there were fewer CD20+ B cells and CD68+ macrophages, but more CD56+ NK cells. Surprisingly, we detected predominantly CD4+ lymphocytes in aGvHD, while in cGvHD, CD8+ cells represented the majority of lymphocytes. CD4+ T cells were mainly found in the dermal compartment, whereas CD8+ T cells located to the area of the epidermal-dermal junction. In cGvHD, the CD8+ lymphocytes stained positive for CXCR3 indicating a Tc1 phenotype, whereas more FoxP3+ CD4+ T cells were found in high grade aGvHD (indicating effector/regulatory T cells). In summary, these results illustrate that a specific immunohistochemical marker profile for skin GvHD can be established. In further examinations, more skin biopsies from patients with GvHD of the skin as well as the main differential diagnoses (drug-reaction, toxic epidermal necrolysis etc.) will be examined immunohistochemically to define markers specific for GvHD.

P120

Interaction between epicutaneously applied ceramides and human stratum corneum

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Ceramides are a unique lipid class that differs substantially from other lipid classes. Because of their bipolar character due to differences of electric charge within the long-chained molecule, ceramides are able to build membrane structures spontaneously. It has become apparent that ceramides are the essential morphological equivalent for the barrier function of the stratum corneum (SC). Significant pathogenetic changes within the barrier function of the SC that are considered to be clinically relevant are known for a variety of dermatoses. To understand the functional structure of ceramides is essential for a clinically effective substitution as well as for the development of pharmacokinetic concepts, i.e. vehicle systems for the transport of active substances into and through the SC. In order to evaluate the integration of substituted ceramides, ceramid-lipid-mixtures have been processed in galenic systems, then the stability and toxicological safety of these systems have been proven and the interaction with human SC *ex vivo* has been investigated using the Franz-diffusion-model. The distribution of the substituted ceramides within the epidermis has been quantified and presented via image analysing methods in two-dimensional pseudo-colour coded tissue sections. The tests have shown that ceramides in special mixtures will be incorporated as so called 'integrationable lipophilic units (ILUs)' into the existing SC structures, and thus, unlike nonpolar lipids, can be integrated directly into membrane structures. The results have prepared the ground for barrier-protective preparations for cosmetic use as well as for the basic therapy in patients with chronic dermatoses with barrier defects.

P121

The epidermal alterations in psoriasis are mediated by IL-22R1 and are amplified by TNF-alpha

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Psoriasis is a chronic T-cell mediated skin disease with a largely unknown pathogenesis, affecting about 2% of the worldwide population. The characteristic psoriatic skin alterations include sharply demarcated, red and slightly raised lesions with silver-whitish scales, which are a result of the massively thickened epidermis with atypical keratinocyte differentiation. In our study we looked for the cause of the epidermal alterations in psoriasis. First, we analyzed the effects of different cytokines on three-dimensional human epidermis. From 10 investigated mediators only IL-20, IL-22, and IL-24 induced acanthosis, parakeratosis and hypogranularity in human epidermis equivalents. The mechanistic studies pointed out that STAT3 activation and down-regulation of K10, CALM5 and DSC1 expressions were involved in these effects. Interestingly, IL-20, IL-22, and IL-24 act via the receptor chain IL-22R1 that is expressed by keratinocytes, but only scarcely by skin fibroblasts and not at all by endothelial cells, melanocytes, subepithelial adipocytes and immune cells. By enhancing the expression of IL-22R1 and its signaling pathway elements, TNF-α enhanced some effects of IL-22R1-using cytokines. Importantly, IL-20, IL-22, and IL-24 are produced by different cells. IL-20 was secreted by activated keratinocytes, IL-22 was produced in particular by CCR6+/CCR4−/CCR10− Th1-cells but also by classical Th22- and Th17-cells and myeloid cells seemed to be the most important source of IL-24. In psoriasis and AD patients, cutaneous IL-20, IL-22, and IL-24 expression was massively increased and correlated with the expression of IL-22R1 target genes. Furthermore, the detailed analyses of cutaneous expression levels of mediators known to activate respective IL-20-, IL-22- and IL-24-producers suggested a network of cytokines (e.g. IL-23, IL-1α) to be involved in induction of these mediators. Interestingly, the IL-20/IL-22 blood level ratios were different between patients pointing that the contribution of individual IL-22R1-using mediators for skin alterations varies between patients. Taken together, our study suggests that IL-20, IL-22 and IL-24 are redundant mediators of skin alterations and IL-22R1 targeting may be a promising therapeutic approach, in contrast to inhibition of single mediators, for the treatment of psoriasis and other chronic-inflammatory skin diseases characterized with overexpression of these cytokines (e.g. atopic dermatitis).

Epidemiology

P122

Demographic data of Adamantiades-Behcet disease patients in Germany (2013) with focus on Turkish and German descent and juvenile and adult onset

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Of the 721 documented patients in the registered charity 'German Registry for Adamantiades-Behet Disease (ABD)' 258 were of German and 308 of Turkish descent along with 30 other countries of origin. The prevalence in Germany is 0.9:100 000 (0.4:100 000 among people of German descent). Manifestation of the disease was predominantly in the third decade of life (median age: 26.5 years). The full clinical picture developed on average in 2.9 years (median 3 months). Most frequent features included oral aphthae (98.5%), cutaneous lesions (81%), genital ulcers (64.7%), ocular manifestations (51.6%), arthritis (52.4%) and positive pathergy test (30.8%). As serious complications blindness 6.8%, meningoencephalitis 4.0%, severe arthritis 2.6%, fatal outcome 1.2%, hemoptysis 1.1% and gastrointestinal perforation 0.5% were registered. The HLA-B5 antigen was positive in 58.1% and showed an association with eye manifestations ($P < 0.001$). The first manifestation influenced the speed of diagnosis: in case of arthropathy, the average interval to diagnosis was significantly extended with 60 months compared to uveitis (15.5 months), superficial thrombophlebitis (13 months) or erythema nodosum (19 months) as onset signs. The evaluation of diagnostic criteria has shown that the International Study Group Criteria for Behet's Disease (1990) have only a low sensitivity of 72.5%

in Germany and that other diagnostic criteria, such as the New International Criteria (2010) with higher diagnostic sensitivity of 88.6% should be taken into account.
Patients of Turkish descent showed androtopism in contrast to those of German descent (female:male 1.9:1), which was also detected in the whole collective (1.4:1). In 12.4% there was a family history with differences between German and Turkish patients (3.8 vs 14.6%; $P < 0.001$) as well as in patients with disease onset in young and adult age (25.0% vs 7.3%; $P < 0.001$). Turkish patients suffered significantly more often from eye manifestations compared to Germans (53.5 vs 43.0%, $P = 0.02$), while in German patients prostatitis/epididymitis (15.3 vs 7.3%; $P = 0.02$) and gastrointestinal involvement (17.1 vs 9.5%; $P = 0.01$) were more frequently documented. The relative risk of HLA-B5 positive individuals is high in both Germans (6.6) and Turks (5.8). In Turkish patients diagnosis was made significantly faster than in German ones (duration from complete clinical picture to diagnosis 1.6 vs 3.1 years; $P = 0.004$).
77 patients (10.7%; among them 35 German and 31 Turkish patients) exhibited the onset of the disease under 16 years of age. Twenty-four patients (3%) exhibited the complete symptom complex before their 16th birthday. In case of onset at juvenile age, ABD seems to exhibit a rather mild monosymptomatic course either with recurrent oral aphthous stomatitis or - less often - with genital ulcers over a period of many years (mean: 7.0 vs 1.4 years in adult onset). Familial history is in particular high in juvenile patients with 25.0% and could indicate individual risk of developing ABD for young patients suffering from recurrent apthous lesions. Recurrent oral aphthae comprised the most common initial sign in young and adult onset (92 vs 83%; $P = 0.04$), recurrent genital ulcers the second common initial sign in both groups (4.0% vs 3.4%; n.s.). In case of young onset ocular manifestations and positive pathergy test were slightly lower, but there were no signicant differences in the frequencies of other organ involvement.

P123

Role of filaggrin gene mutations and atopy in severe occupational irritant contact dermatitis of the hands

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Loss-of-function mutations in the gene encoding filaggrin (FLG) have been associated with atopic dermatitis and may also influence the prognosis of irritant contact dermatitis (ICD). In this prospective cohort study, we evaluated the clinical course, recovery rate, periods away from work and job continuation in 459 patients with occupational ICD of the hands taking part in a 6-week inpatient/outpatient rehabilitation program conducted by the University of Osnabrueck. The patients came from different occupational backgrounds. Follow-up visits were organized for up to 3 years. Patients were genotyped for four common FLG mutations and examined for atopy. A total of 327 (71.2%) of the recruited patients were considered as atopic (A) and 132 as non-atopic (NA). Overall, 68 patients (A: $n = 60$ and NA: $n = 8$) had a mutation in the FLG alleles R501X, R2447X, S3247X and 2282del4. Non-atopic patients with ICD responded better to therapeutic approaches, while atopic patients with ICD had more resistant lesions, resulting in a higher use of topical corticosteroids as well as lower rates of recovery and job continuation. The presence of FLG loss-of-function mutations worsened the course of occupational ICD, but only in combination with atopy, not as an independent risk factor. After 3 years, the rate of those abandoning the profession was significantly increased in atopic patients with ICD and FLG mutations compared to non-atopic patients with ICD and no FLG mutations (OR 3.1; $P = 0.047$). In conclusion, FLG mutations seem to modify the course of occupational ICD, but only in the presence of atopy. Identification of this subgroup at risk may help to implement specific preventive measures at an early stage.

P124

Incidence and determinants of chronic pruritus: a population-based cohort study

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Background: Pruritus is the most common symptom in dermatology and frequently occurs in a range of other conditions. Epidemiological data on chronic pruritus (>6 weeks) in the general population is sparse. Recent surveys suggest a considerable prevalence of chronic pruritus and have identified a number of determinants cross-sectionally. We aimed to provide first data on the incidence of CP, additional data on its prevalence, and examine longitudinal associations of the symptom with sociodemographic variables and conduct a comprehensive analysis of cross-sectional associations with chronic diseases, lifestyle and psychosocial variables.
Methods: A cohort of 1190 participants from a cross-sectional baseline-study was followed-up after 1 year. The questionnaire assessed occurrence of the symptom, medical variables, lifestyle and psychosocial variables. Incident chronic pruritus was defined as reported pruritus at follow-up in those free of the symptom at baseline. Cross-sectional analyses of data from the follow-up assessments addressed potential associations of medical variables, lifestyle and psychosocial factors with prevalent chronic pruritus. Longitudinal analyses examined sociodemographics as potential predictors of incident chronic pruritus. Robust Poisson-regression analyses were conducted.
Results: The follow-up response-rate was 83.1%. The 12-month cumulative-incidence equalled 7.0% [95% confidence interval (CI): 5.2–9.2%]. The lifetime-prevalence was 25.5% (CI 21.8–27.8). Incidence was significantly associated with age. Incidence was significantly associated with age. Determinants of prevalent CP in multivariable-analyses were liver disease, asthma, eczema and dry-skin within the medical-domain, an elevated Body-Mass-Index within the lifestyle-domain and higher anxiety-scores within the psychosocial-domain.
Conclusions: This is the first study investigating the incidence of chronic pruritus and its determinants at the population level. Findings suggest a considerable 12-month-incidence and lifetime-prevalence and provide important directions for future research. Knowledge about the risk factors for chronic pruritus may inform the development of preventive interventions and allocate resource to where they are needed.

P125

Psychometric properties of the DLQI: applying the Rasch model in a sample of hand eczema patients

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Background: Hand eczema (HE) is often a chronic or recurrent disease and leads to a substantial loss of Health-related Quality of Life (HrQoL) over extended periods of time. The Dermatology Life Quality Index (DLQI) developed by Finlay et al. (1994) is a 10-item skin-disease-specific HrQoL measure, and represents the most frequently used HrQoL instrument in dermatology. To date, its psychometric properties in HE patients have only been explored by applying the principles of classic test theory.
Objectives: To explore the psychometric structure of the DLQI in a sample of patients with HE using modern test theory by applying the Rasch model.
Methods: We explored HrQoL impairments in a sample of $n = 602$ patients with HE. After applying the coding rules from the authors of the DLQI $n = 527$ were eligible for analysis (mean age 47.0 years, SD 11.6; 37.4% males, 62.6% females). The patient sample was drawn from two inpatient hospitals providing a tertiary prevention program in Germany. The data was analyzed using RUMM2030. We retrieved overall fit to the Rasch model by testing the item-trait interaction and the person separation index (PSI) was used as measure of internal reliability. On item level the DLQI was inspected according to category frequencies, thresholds, differential item functioning (DIF) and fit residuals.
Results: The DLQI had a good PSI of 0.82 but misfitted the Rasch model significantly (mean item interaction 0.00, SD 0.7; mean person interaction −0.86, SD 1.3; item-trait interaction $P < 0.000$). A more detailed examination on item level revealed that four items showed significant misfit with fit-residuals outside the 2.5 range indicating that those items represent an overfit (negative values) to the scale (adding only little information to the scale) or overdiscrimination (positive values; are poorly associated with the scale). Besides that seven items showed uniform DIF according to gender or age groups, two items showed non-uniform DIF between centers or age groups and two items had disordered thresholds. A recalibration of the DLQI using the Rasch model was successful, but resulted in a strongly reduced scale with six items and a maximal score range from 0 to 17.
Conclusions: The use of the DLQI in HE cannot be recommended. If the DLQI is used in an ongoing study we suggest using the alternative scoring we have retrieved by applying the Rasch model – all items concerning personal relationships had to be removed from the scale, this has to be accounted for while interpreting the data. To asses impairments in HrQoL of HE patients a new disease-specific instrument is needed.
Reference: Finlay A Y, Khan G K. Dermatology Life Quality Index (DLQI) – a simple practical measure for routine clinical use. Clin Exp Dermatol 1994.

P126

Withdrawn

P127

Prevalence of acne, obesity and hyperandrogenism in patients with Down syndrome

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Background/Objective: Acne vulgaris is a multifactorial disease of pilosebaceous unit with genetic background. It exhibits a worldwide prevalence around 95% among adolescents. Its etiopathogenesis involves increase in sebum production and follicular hyperkeratinization; follicular colonization by Propionibacterium acnes which stimulate secretion of cytokines and inflammation. Down syndrome (DS) is the most common autosomal chromosomal disorder. Tendency to obesity in individuals with DS is well-known with a greater prevalence among females. Diet, lack of physical exercise and a possible role of hormones related to obesity, such as leptin, have been considered. A higher prevalence of diabetes has been reported in DS compared to general population and, although it can appear as autoimmune disorder it is highly prevalent as consequence to increased insulin resistance. Additionally, increase in body weight is accompanied of elevations in cardiovascular risk markers. There are no data on the association of DS with acne, obesity and hyperandrogenism. Our aim was to detect the prevalence of acne, its forms and associated factors and analyze the occurrence and severity of obesity, explore its impact on arterial blood pressure, inflammatory markers and cardiovascular risk factors in DS.
Method: Cross-sectional study including 89 subjects aged 10–28 years from a Brazilian specialized healthcare center for DS (APAE-Sao Paulo) was conducted to verify acne, metabolic and hormonal disorders by interview, clinical and laboratory examinations. The study was approved by Institutional Review Board and subjects or a responsible person signed informed consent. Patients were screened for obesity, arterial blood pressure, waist abdominal circumference and collected blood for inflammatory, endocrine and lipid profile. Database collected from subsequent visit screening included gender, age and race, weight, height and waist measurements, Body Mass Index (BMI) and BMI standard deviation score calculation, and drugs taken at that time. Blood analysis included glucose, fasting insulin, HOMA index, C reactive Protein (CRP), total cholesterol (Tc), HDLc, LDLc, triglycerides and VLDLc. Testosterone, FSH, S-DHEA, LH, LH/FSH analysis were performed for girls to exclude hormonal causes of obesity.
Results: We evaluated 49 (55%) males and 40 (45%) females. Prevalence of acne was 70.8%, 83.7% in males and 55% in females and for age groups 10–17 and 18–28 was 62% and 78.7%, respectively. That was lower than prevalence (96%) detected in 452 adolescents from four schools in Sao Paulo city, aged 10–17, with no difference between males (95.9%) and females (96.1%). Facial comedonal acne was mostly observed (65%) as well as for adolescents from Sao Paulo city (62%). The prevalence of obesity was 40%, metabolic disorders 7% and hyperandrogenism (females) 15%. Except for gender, no other factor evaluated correlated with acne. According to metabolic analysis, BMI and CRP were the most often altered variables. Comparatively, in the group aged less than 19, girls have higher insulin values and HOMA index than boys. In group aged 19 or more, boys had higher systolic and diastolic blood pressure, waist circumference, Tc and LDLc compared to girls of same age. There was also a strong association between changes in blood pressure levels and BMI, Tc and insulinemia.
Conclusion: Low prevalence of acne in Down syndrome, predominance in males aged 18–28 and facial comedonal form were detected. Association with obesity, metabolic disorders was not detected, although higher prevalence of metabolic alterations were observed.

Genetics

P128

Functional molecular-genetic analysis of 16 XP-C patients from Germany: environmental factors predominately contribute to phenotype variations

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Patients belonging to Xeroderma pigmentosum (XP) complementation group C comprise one third of all XP patients. Only four major reports compiled larger groups of XP-C patients from southern Europe (12 pts), North America (16 pts), and Africa (14 pts and 56 pts) as well as their genetic background (46 XPC mutations). We identified 16 XP-C patients from Germany. Interestingly, only five patients exhibited severe sun sensitivity. The mean age of XP diagnosis was 9.4 years, the median age of the first skin cancer was 7 years. Neurological symptoms were absent in all but two patients. Primary fibroblasts from all 16 patients showed reduced post-UV-cell survival (mean: 50% vs 93% in normal cells) and reduced reactivation of an UV-treated luciferase reporter gene (mean: 4.9% vs 30.7% in normal cells). XPC mRNA expression was also greatly reduced compared to normal cells (mean: 15.3%; range 8.3–25.7%) except in XP47MA (274.13%). All patients carried homozygous XPC

mutations. Four mutations have been described previously: c.1747_1748delTG (found in 4/16), c.567C>T (4/16), c.1839 C>T (1/16), and a complex insertion/deletion mutation in exon 9 (1/16). The novel frameshift mutations c.446_447delAG (2/16), c.1525insA (1/16) and c.2271delC (1/16) lead to truncated XPC proteins as does the novel nonsense mutation c.843C>T (1/16). XP47MA carries an interesting mutation (c.2538_2540delATC; p.Ile812del) resulting in an in-frame single amino acid deletion. This mutation results in a classical XP phenotype, a non-functional XPC protein, but elevated XPC mRNA expression. Our study indicates that extrinsic factors may contribute the most to XP-C symptom severity due to nonsense-mediated message decay and non-functional, mostly truncated proteins.

P129

Low incidence of oncogenic EGFR, HRAS and KRAS mutations but absence of FGFR2, PIK3R1 and NRAS mutations in seborrheic keratosis

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Seborrheic keratosis (SK) represents a frequent epidermal skin tumor. Although lacking a malignant potential, these tumors reveal multiple oncogenic mutations. A previous study identified activating mutations in 89% of SK (Hafner et al., PNAS 2010), particularly in FGFR3 and PIK3CA genes. The aim of this study was to identify further oncogenic mutations in human SK. Therefore we screened for mutations in EGFR, FGFR2, PIK3R1, HRAS, KRAS, and NRAS genes using both Sanger sequencing of selected exons as well as a multiplex SNaPshot assay in 58 SK of 14 patients. We identified a somatic EGFR p.L858R mutation in one SK. Furthermore, the HRAS mutations p.G13R (2/58 SK) and p.Q61L (2/58 SK) were found. These mutations have not been described in human SK yet. In addition, one SK revealed the KRAS p.G12V mutation, which has already been reported in SK. No mutations were detected in FGFR2, PIK3R1 and NRAS genes. The results of this study suggest that activating mutations of EGFR, HRAS and KRAS contribute to the pathogenesis of human SK, though at a lower frequency than FGFR3 and PIK3CA mutations. FGFR2, PIK3R1 and NRAS mutations obviously do not have a significant role in the development of SK.

P130

Nevus marginatus: a combined organoid and non-organoid epidermal nevus caused by HRAS mutation

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Nevus marginatus is a peculiar epidermal nevus characterized by the combination of a central organoid (sebaceous) component and a bordering non-organoid (keratinocytic) component. The pathogenesis of nevus marginatus remained unknown. Mosaic RAS mutations have been previously reported as the genetic cause of both sebaceous nevi and keratinocytic epidermal nevi. We analyzed DNA isolated from nevus marginatus and adjacent normal skin for RAS mutations. The HRAS c.37G>C (p.Gly13Arg) mutation was identified in the sebaceous part, the keratinocytic part and in a common wart that had developed within the nevus marginatus. Adjacent normal epidermis revealed a wild-type sequence at HRAS codon 13. Our results indicate that nevus marginatus is caused by a mosaic HRAS mutation. In congruence with previous genetic analyses of keratinocytic epidermal nevi and sebaceous nevi, this study suggests that a postzygotic HRAS mutation of a multipotent epithelial progenitor cell can result in both organoid and non-organoid epidermal nevi, depending on its differentiation potential. Nevus marginatus represents a paradigm for this concept.

P131

No association of STAT3 rs4796793 SNP with response to IFNα therapy in patients with metastatic melanoma

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Interferon alpha (IFNα) is approved for the adjuvant treatment of stage III melanoma patients in Europe and USA. The efficacy is, however, unfortunately restricted to a subpopulation of patients while side effects are common in most of the treated patients. Thus, the identification of predictive biomarkers would be highly beneficial to select patients for IFNα therapy. In this regard, the STAT3 rs4796793 SNP has recently been reported to be associated with IFNα sensitivity in metastatic renal cell carcinoma. Thus, we analyzed the impact of this SNP on the clinical outcome of 322 stage III melanoma patients of which 130 had received adjuvant IFN treatment. This analysis did not reveal any significant association between the STAT3 rs4796793 SNP and patients' progression free or overall survival. Moreover, although previously reported for immortalized B-cell lines, we did not detect a correlation between SNP genotype and STAT3 mRNA levels, neither for melanoma cells nor for peripheral blood lymphocytes. Interestingly, there was a trend that melanoma cells carrying the minor allele were more sensitive to IFNα *in vitro*; however, this difference was not statistically significant. In conclusion, based on our findings the STAT3 rs4796793 SNP is no predictive marker for the efficacy of adjuvant IFNα treatment in melanoma patients.

P132 (O18)

In variegate porphyria, inactivation of both alleles of the protoporphyrinogen oxidase gene by null mutations gives rise to hepatocellular carcinoma

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Variegate porphyria (VP) is an acute hepatic porphyria that results from an autosomal dominantly inherited deficiency of protoporphyrinogen oxidase (PPOX), the seventh enzyme in heme biosynthesis. Affected individuals can develop both cutaneous and acute neurovisceral symptoms. A rare, but serious complication in VP and other acute hepatic porphyrias is the development of hepatocellular carcinoma (HCC). HCC is the sixth most common cancer in the world and a leading cause of death in many countries. Several well-defined viral and environmental risk factors contribute to the development of HCC but as of yet, the pathomechanisms underlying hepatocarcinogenesis in VP largely remain elusive. Here, we sought to elucidate if PPOX could have a hitherto unknown function as a tumor suppressor. After obtaining postmortem cancerous and non-cancerous liver tissue from a female VP patient with HCC we first identified the underlying PPOX germline mutation, c.1082–1083insC, which results in a null allele. Interestingly, in the tumorous liver sections we also detected a somatic second hit mutation, designated c.K416X, in trans to the germline mutation, which likewise encodes a null allele. This second hit was absent in non-tumorous liver tissue and various other organs studied for control purposes. By reverse transcriptase PCR-analysis we could only detect the allele linked to the germline mutation, suggesting nonsense-mediated mRNA decay as the result of the trans-acting somatic mutation in the tumor. Immunohistochemistry revealed almost complete absence of PPOX staining in the cancerous liver tissue, confirming our molecular genetic findings on the translational level. Our data suggest that PPOX plays a crucial role in tumorigenesis of HCC associated with VP by acting as a tumor suppressor. Based on these findings, we for the first time propose a mechanistic model for the development of HCC in VP that could probably also be applied to other acute hepatic porphyrias.

P133

Dual porphyria comprising porphyria cutanea tarda and acute intermittent porphyria without cutaneous symptoms

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The simultaneous dysfunction of two enzymes within the heme biosynthetic pathway in a single patient is rare. To date, only 15 cases have been reported. A 60-year old Caucasian woman of German origin had a medical history of recurrent colicky abdominal pain attacks, nausea, vomiting, diarrhea, paresthesia of the upper and lower extremities, and episodes of paralysis. Biochemical analyses showed elevated total porphyrins and an increase of the both porphyrin precursors delta-aminolevulinic acid and porphobilinogen in the urine. The tentative diagnosis of acute intermittent porphyria (AIP) was confirmed by molecular genetic analysis and identification of a heterozygous germline mutation in the porphobilinogen deaminase (PBGD) gene, designated c.517C>T (p.R173W). Interestingly, we also noted an elevation of stool porphyrins, which is rather uncommon in AIP. Further stool porphyrin differentiation revealed isocoproporphyrin in the feces, which is the biochemical hallmark of porphyria cutanea tarda (PCT) and has as of yet not been described in any other type of porphyria. Therefore, we made the diagnosis of a dual porphyria comprising AIP and PCT. Both AIP and PCT can be associated with complicating liver disease such as hemosiderosis, cirrhosis and hepatocellular carcinoma. Thus, patients diagnosed with either one of these porphyrias should have a regular bi-annual screening of their liver function by blood analysis and liver ultrasound. Our patient revealed as hitherto unrecognized multiple liver cysts that require further follow-up. Considering the presence of fecal isocoproporphyrin, the unusual aspect of this patient is that she has never before experienced cutaneous symptoms.

P134

Mutations of IL36RN in four patients with generalized pustular psoriasis of German, Turkish and Iraquian origin

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Generalized pustular psoriasis (GPP) is a severe skin disease that is characterized by repeating flare-ups of pustular psoriasis and multisystemic inflammation. Recently, missense mutations of IL36RN have been identified in GPP patients from Tunisia and Europe. We screened a group of 12 patients recruited at five German university hospitals for IL36RN mutations; 10 patients showed a typical GPP phenotype, phenotypes of the other two resembled GPP in some aspects. We identified the previously undescribed homozygous missense variant c.227C>T/ p.Pro76Leu affecting a highly conserved nucleotide in a Turkish patient of a consanguineous couple. Molecular modeling revealed a reduced stability of the protein as well as local structural rearrangements, indicating its disease-causing nature. Two further patients were either compound heterozygous for the two European mutations p.Arg48Trp and p.Ser113Leu, or p.Ser113Leu and the newly identified stop mutation p.E94X, one further proband was homozygous for p.Ser113Leu. No mutations were identified in the two patients with a phenotype resembling GPP. Carriers of mutations had an averaged earlier age of onset than non-carriers. Our findings support the role of IL36RN in the pathogenesis of GPP, while mutations p.Arg48Trp and p.Ser113Leu seem to be specific to European populations. The rate of 40% IL36RN mutation carriers of our study provides further evidence that GPP is a heterogeneous disease. Therapy with a recombinant interleukin-1 receptor antagonist in some GPP patients is effective, which supports the role of IL36RN mutations as disease-causing factors in GPP and underlines the importance to reveal the molecular basis of GPP in single patients.

P135 (O17)

A mutational storm revealed by Exome sequencing: Dramatic increase of UV signature mutations during development of skin cancer in Xeroderma pigmentosum patients

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Xeroderma pigmentosum (XP) is a rare autosomal recessive disease clinically characterized by photosensitivity, xerosis cutis, poikiloderma, telangiectasia and a 1000-fold increased risk to develop skin cancer. Patients with XP are defective in nucleotide excision repair (NER) a mechanism responsible for removal of bulky helix-distorting DNA damage mainly induced by ultraviolet (UV) radiation. When UV-induced DNA damage is not removed from the genome, the remaining photoproducts will give rise to UV-signature mutations such as C to T and CC to TT transitions. While in XP patients it has been shown that isolated genes such as p53 harbor such mutations, thus far it was technically impossible to comprehensively investigate the exome of the whole human genome. This is the first study analyzing the complete exome of humans in this context. Here, we identified somatic mutations in DNA from three patients, suffering from in Germany the most frequent XP complementation group C. Skin samples of non-sun-exposed, sun-exposed and manifest skin tumors at UV-exposed body sites were compared to the respective germline genomes derived from patient blood as well as samples from individuals with normal DNA repair. Exome-sequencing was performed using sequence capture (Agilent Human All Exon 50Mb Kit) followed by next-generation sequencing using an Illumina Genome Analyzer II with subsequent data analysis allowing for an average exome coverage of >50 reads per base and a total target coverage of >90%. The target region covered 36 Million base pairs, including 30 000 genes. Subsequently, we compared mutation levels of sun protected tissues against the mutations of the sun exposed tissues or tumor tissues. The mutations which were only present in sun exposed or tumor tissue are called differential mutations. When we looked for differential mutations in XP patients, we found a dramatic increase of the mutational load, of which a high percentage were UV fingerprint mutations (transitions). This dramatic mutational load was not observed in control groups of elderly people. The differential mutation level in skin cancers of XP patients was also extremely high. When compared to sun

protected tissue of XP patients. A total of 4642 mutations of which 98% were transitions were exclusively identified in tumor tissue. Importantly, this high mutational load was not observed in other tumors of non XP patients.
Interestingly the highly elevated cancer risk in XP patients correlates well with the excessive load of differential mutations in the tumors of the patients. These findings point to a direct link of UV induced mutational load and skin cancer.
Annotation analysis will reveal relevant pathways with UV signature mutations to carcinogenesis.
These findings can contribute to solving the questions of the existence and magnitude of mutational threshold levels sufficient for cancer formation.

Health Services Research

P136

Disease activity only moderately correlates with Quality of life Impairment: results of the Greek validation of the Chronic Urticaria Quality of Life Questionnaire (CU-Q2oL)

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Background: Chronic spontaneous urticaria (csU) has a remarkable impact on quality of life of patients. Because the disease is hard to treat the treatment goals should be defined, which requires validated instruments to approach disease activity.
Objective: The aim of this study was the validation of the Greek CU-Q2oL and the use of this instrument in order to access the correlation of disease activity, as approached by urticaria activity score (UAS), with quality of life impairment.
Methods: After forward and back translation, according to the guidelines, the Greek version of the CU-Q2oL was applied to 110 Greek csU patients along with the UAS. To access the scale structure of CU-Q2oL, factor analysis was applied and internal consistency was calculated. To access the relation between disease activity and quality of life impairment correlations were calculated and multiple regression analysis was performed.
Results: Exploratory factor analysis revealed a six scale structure of the Greek version of CU-Q2oL, i.e. 'Functioning', 'Sleep', 'Embarrassment', 'Eating/Limits', 'Mental status' and 'Symptoms' that explained 67.9% of its total variety. The internal consistency was satisfactory with Cronbach's $\alpha > 0.7$ for each scale. Disease activity, determined by the UAS, was found to be only moderately correlated with the CU-Q2oL total score ($r = 0.40$, $P < 0.0001$). In order to investigate the ability of CU-Q2oL to discriminate between different severely affected csU patients, we created three UAS groups (group 1: 0–12, group 2: 15–22 and group 3: 23–42). Subsequently, we computed the quality of life impairment. There was a statistically significant difference of the CU-Q2oL total score between groups 1 and 2, but not between groups 2 and 3. Disease activity in contrast to age, sex and disease duration was found to be predictor of quality of life impairment ($P < 0.01$).
Conclusion: The Greek CU-Q2oL is a valid and reliable instrument that can be used in research but also in every day clinical practice. To obtain a comprehensive picture of the disease status of patients with chronic spontaneous urticaria, both the CU-Q2oL and the UAS should be applied.

P137

Self-assessed disease severity is a powerful predictor of health-related quality of life in chronic hand eczema

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Background: Little is known about predictors of health-related quality of life (HrQoL) in chronic hand eczema (CHE). It was the aim of this study to analyse potentially predictive factors in patients with CHE, using data from the German carpe (German acronym: Chronisches Handekzem-Register zum Patienten-Langzeitmanagement) registry.
Methods: In the carpe registry, patients with chronic hand eczema (CHE) are assessed by means of a dermatological examination and a patient questionnaire (1). Medical history, clinical and demographic characteristics as well as characteristics of therapy and health care are assessed. Health-related quality of life (HrQoL) is measured by the Dermatology Life Quality Index (DLQI) which consists of ten items (2).
The following variables were entered in blocks into a hierarchical linear regression model: socio-demographics (age, gender, body mass index (BMI)), disease-related variables (Physician Global Assessment (PGA), duration of CHE, other body areas affected, feet affected, number of hands affected, itch, treatment-related variables (topical treatment, phototherapy, systemic treatment), predisposition (atopic skin diathesis), health care utilization (GP visits, dermatologist visits) and experience of treatment (treatment burden, treatment time consuming, unpleasant side effects, satisfaction with care, application of treatment recommendation) and self-assessed disease severity.
Multivariable linear regression analysis was used to identify factors which were independently associated with HrQoL. Standardized β coefficients were computed using SPSS for Windows. Adjusted R square was computed as a measure of explained variance.
Results: A total of 1013 patients (53.7% female, mean age: 47.6 years) with CHE were eligible for this predictive analysis. The DLQI had a mean of 8.82 (SD = 6.31) and a median of 8.00 ($N = 992$).
In the final model after all variables had been entered simultaneously ($N = 582$), the strongest significant predictor was self-assessed CHE severity ($\beta = 0.38$), followed by having experienced unpleasant side effects ($\beta = 0.16$), general treatment burden ($\beta = 0.11$), physician global assessment ($\beta = 0.11$), atopic skin diathesis ($\beta = 0.09$) and having visited a GP ($\beta = 0.09$). Age, gender, BMI, duration of disease, other body areas affected, number of hands affected, feet affected, itch, treatment, having visited a dermatologist, time consuming treatment, satisfaction with care and being able to apply treatment recommendation did not emerge as significant predictors in the final model. A 39% of the variance were explained by the joint variables.
Conclusions: Self-assessed disease severity is a powerful predictor of HrQoL in CHE. Unpleasant side effects require special attention in the care of patients with CHE. Demographic variables, itch, localisation and duration of CHE as well as type of treatment received did not predict HrQoL.
A 61% of the variance remains to be explained suggesting that unexplored interactions as well as yet unknown factors need to be identified.
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Pressure ulcer prevention: performance of the Braden scale in ICU and NCU

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Background: Pressure ulcer prevention is a relevant public health goal due to the substantial clinical and economic burden of pressure ulcers. Therefore, the Braden Scale is a frequently applied scale for measuring the risk to develop a pressure ulcer.
Aim: Evaluation of the pressure ulcer documentation in the university hospital Carl Gustav Carus Dresden, Germany (UKD). Comparison of the performance of the Braden Scale in intensive care units (ICU) and normal care units (NCU).
Method and Materials: Longitudinal study including all inpatients treated at the UKD between 2007 and 2011 ($n = 246\,162$; 48.4% female and 41.6% male; mean age 49.6 years). Documentation of pressure ulcer risk by means of the Braden Scale and clinical signs of pressure ulcers at admission, weekly follow-up examinations, and at discharge by trained staff. Primary outcome was incident pressure ulcer during inpatient treatment. ROC curve analysis was applied to evaluate the performance of the Braden Scale in NCU versus ICU. The area under curve (AUC) is equal to the probability that a classifier will rank a randomly chosen positive instance higher than a randomly chosen negative one.
Results: The overall incidence of pressure ulcers during inpatient treatment was 0.78%. As expected, a higher rate of pressure ulcers was observed at ICU versus NCU (4.77% vs 0.59%). At the proposed cutoff of 18, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the Braden Scale at NCU were 44.57%, 89.95%, 3.14% and 99.69%. In contrast, at ICU, sensitivity, specificity, PPV, and NPV of the Braden Scale were 96.61%, 28.46%, 5.86% and 99.24%.
The area under the ROC curve as an indicator for the overall performance of the Braden Scale was 84.89% at NCU versus 69.00% at ICU.
Discussion: This validation study based on data of a very large prospective study indicates that the Braden score is an appropriate instrument to determine the risk for incident pressure ulcer in normal care units, whereas its performance in intensive care units is not as good. Therefore, alternative instruments need to be developed to determine the risk for incident pressure ulcers in the ICU setting.

P139

The role of mental health on the impact of atopic disease on children and adolescents' health related quality of life?

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Background: Eczema, hay fever and asthma are global health problems and have been linked to decreases in health-related-quality-of-life (HRQoL) in adults and children/adolescents. Research also suggests an association of the three conditions with mental health, which in turn is related to HRQoL decreases. However, no data is available with respect to whether the level of HRQoL impairment varies as a function of mental health problems in children and adolescents suffering from atopic disease. We aimed to assess whether the impact of any of the three conditions on HRQoL is modified by the presence of mental health problems.
Methods: The impact of occurrence of the three conditions within the past 4 weeks on HRQoL, as measured by KINDL-R was analysed by use of the complex sample general linear model in a population-based sample ($N = 6518$) of children and adolescents aged 11–17. Analyses were adjusted for the respective other atopic conditions, sociodemographic and clinical variables and stratified for mental health as measured by the Strengths and Difficulties Questionnaire (normal $n = 5697$, borderline $n = 609$, abnormal $n = 193$).
Results: Eczema was significantly associated with decreased total KINDL-R scores and the physical and self domains after adjusting for all other variables when no mental health abnormalities were present. Hay fever within the past 4 weeks was significantly associated with decreased total scores and the physical domain after adjusting for all other variables when no mental health abnormalities were present. Asthma was associated with better HRQoL in these individuals but only the school domain was significant. However, when mental health problems were present, eczema was positively associated with several subscales and the positive impact of asthma was stronger. The presence of mental health problems accentuated the negative relationship between hay fever and HRQoL (stronger negative impact). However, due to decreasing numbers in the group with mental health problems only few associations reached statistical significance.
Conclusions: The results suggest mental health to have a modifying effect on the relationship between atopic conditions and HRQoL. When mental health problems are present eczema and asthma are associated with better HRQoL while hay fever was associated with worse HRQoL. However, caution needs to be exercised in interpreting the results as the groups with borderline or abnormal mental health were comparably smaller than the group with normal mental health. In the group with normal mental health small effects were more likely to become significant than in the other two groups.

Immunology

P140

Regulation of pro-and anti-inflammatory Th17 cell responses by microbial organisms

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Th17 cells emerged as a new T helper cell lineage several years ago which is involved in the clearance of extracellular bacteria and fungi. A dys-regulated Th17 response, however, can induce severe tissue destruction and autoimmunity. Therefore, mechanisms must be in place to shield the host from immune-mediated damage.
We demonstrate that human Th17 clones can produce IL-10 upon stimulation with a delayed kinetics. Interestingly, IL-10 expression was accompanied by reciprocal down-regulation of IL-17, leading to a functional regulatory Th17 cell phenotype after the peak of the immune response. The ability of Th17 cells to express IL-10 was restricted to certain pathogen specificities. *Ex vivo* isolated *C. albicans* specific Th17 cells could not produce IL-10 in comparison to *S. aureus* specific Th17 cells. This was due to differential priming requirements of these Th17 cell sub-populations. IL-1beta instructed naive T cells to develop into a pro-inflammatory non-IL10 expressing Th17 cells subset. Th17 cell priming with *S. aureus*, however, was not IL-1beta dependent, leading instead to the generation of IL-10 producing Th17 cells with self-regulatory activities.
Our results identify pathogen dependent differential priming requirements for human Th17 cells and demonstrate that IL-1beta has a switch factor role in determining a functional memory for IL-10 expression. This has important consequences regarding the physiological termination of pro-inflammatory immune responses and the limitation to bystander damage in certain pathogen microenvironments. Targeting IL-1beta early in the differentiation process of Th17 cells might therefore represent a promising therapeutic strategy to confer anti-inflammatory properties to the main cellular mediators of autoimmune diseases.

P141

Aberrant expression of heat shock protein 90 in skin and blood of bullous pemphigoid patients

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The cell stress chaperone heat shock protein 90 (Hsp90) has been implicated in inflammatory responses and its inhibition has proven successful in different mouse models of autoimmune diseases, including epidermolysis bullosa acquisita. Here, we investigated expression levels and secretory responses of Hsp90 in bullous pemphigoid (BP) patients and healthy controls. Compared with controls ($n = 32$), an increased intracellular accumulation of Hsp90 was detected in perilesional skin biopsies and extracts of peripheral blood mononuclear cells of BP patients ($n = 12$) by fluorescent immunohistochemistry and ELISA, respectively. In contrast, Hsp90 serum levels were lower in BP patients than in healthy subjects as measured by ELISA. Interestingly, intraindividual serum levels of Hsp90 increased in parallel with both healing of skin lesions and decline in circulating anti-BP180 NC16A antibodies. To investigate if comparatively higher intracellular and lower serum levels of Hsp90 in BP patients are a result of compromised secretion of this protein into the extracellular space, peripheral blood mononuclear cells were cultured and stimulated by the proinflammatory agents TNF-alpha and LPS/CpG. In fact, Hsp90 levels in supernatants from stimulated cell cultures of BP patients were lower compared to those of controls as measured by ELISA. Similarly, ELISA measurements of the Hsp90 content in conditioned medium of HaCaT cells revealed reduced levels of this protein after stimulation of the cells with affinity-purified anti-BP180 NC16A IgG compared to incubation with total IgG of normal human serum from a healthy blood donor. By ELISA and immunoblotting, these effects were not due to discriminative expression of TGF-alpha, protein kinase A and protein phosphatase 5, all of which were previously described to be involved in the regulatory mechanism of Hsp90 secretion. In sum, our results reveal an upregulated Hsp90 expression at site of inflammation and an autoantibody-mediated dysregulation of the intracellular and extracellular distribution of Hsp90 in BP patients compared to healthy subjects, thus suggesting a pathophysiological role of this protein in this autoimmune bullous disease.

P142

IFN-α disarms human CD4+CD25+FOXP3+ regulatory T cells through cAMP repression without alteration of the Treg lineage program

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IFN-α acts as treatment of viral infections and malignancies, including malignant melanoma and cutaneous lymphomas. Previously, we have shown that IFN-α abolishes suppressor activity of human CD4+CD25high FOXP3+ regulatory T cells (Treg) *in vitro* and *in vivo* in a humanized xenogenic GVHD mouse model. Notably, disarming of Tregs by IFN-α was accompanied by cAMP repression. Therefore, the aim of this study was to figure out the effect of IFN-α on the properties of human Treg with focus on cAMP-related pathways. As cAMP levels are controlled by phosphodiesterases-mediated degradation, we used the non-selective phosphodiesterase inhibitor IBMX and the PDE4 (highly expressed in T Cells) specific inhibitor rolipram. In both settings, restoration of cAMP amounts renewed the suppressive functions of human Tregs, emphasizing the functional relevance of IFN-α-induced reduction of cAMP in Treg. Activation of PDE4 in T cells is regulated by the MAP kinases Erk1/2. Blocking of this pathway by an Erk-specific inhibitor in IFN-α-treated Treg completely restored cAMP accumulation, indicating that IFN-α repressed cAMP levels through modulation of Erk pathways that subsequently regulated PDE4 activity. Recently, a conserved CpG-rich element within the FOXP3 locus was found to be demethylated in Treg and that this Treg-specific demethylated region (TSDR) was associated with transcriptional activity and a stable imprinted phenotype of Treg. However, bisulfite pyrosequencing of the TSDR revealed unchanged methylation levels in resting and activated Treg upon IFN-α incubation. In addition, we did not observe an alteration of the anergic state and the cytokine profile of human Treg by IFN-α treatment. Thus, our data indicate that IFN-α did not influence the Treg differentiation program. In conclusion, our study demonstrates that IFN-α interferes with the suppressive activity of human CD4+CD25+FOXP+ Treg by affecting cAMP regulation through MAP kinase/PDE4-mediated pathways while maintaining the Treg lineage program, suggesting a transient Treg inhibition as an important mechanism in IFN-α-mediated immune regulation.

P143 (O09)

Inflammatory gammadelta T cells critically contribute to psoriasiform dermatitis in the CD18hypo PL/J mouse model

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The contribution of Interleukin-17 (IL-17) to the pathogenesis of psoriasis is substantiated by the clinical efficacy of antibodies against the common IL-12/IL-23 subunit p40, like Ustekinumab, suppressing IL-17 and Th1 cytokine production. To further address the significance of distinct IL-17 producing T cell subsets in psoriasis, CD18hypo PL/J mice, developing a psoriasiform dermatitis at 12–14 weeks of age as a consequence of reduced expression of CD18/β2 integrin to 2–16% of wildtype levels, were systematically analyzed for the presence of gammadelta and CD4+ T cell subsets in blood, lymphnodes and skin and the effect of blocking different targets within the IL-23/IL-17 axis. Severity of CD18hypo PL/J psoriasiform dermatitis generally correlated with a loss of skin-resident Vγ5+ T cells and concurrent skin infiltration with inflammatory gammadelta TCRlow expressing Vγ4+ T cells preceded by an increase of IL-23R+ Vγ4+ T cells in local lymphnodes in this psoriasis mouse model. Injection of diseased CD18hypo PL/J mice with anti-$\gamma\delta$ TCR-, -IL-17, and -IL-23 antibodies resulted in an almost complete resolution of skin inflammation and eliminated pathological Vγ4+ T cells. In peripheral blood and skin sections from psoriasis patients we observed a variable increase in $\gamma\delta$ T cells that correlated with a reduction of CD18 levels compared to age-matched healthy subjects. These results for the first time demonstrate a critical role of skin-infiltrating inflammatory Vγ4+ T cells in a complex psoriasis model as well as a role for wildtype CD18 expression levels in suppression of pathological $\gamma\delta$ T cells and suggest the need for individualized therapy of psoriasis patients depending on the skin infiltrate.

P144

Mast cells control skin inflammation in a chronic allergic contact dermatitis model in mice

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Mast cells (MCs) have been shown to modulate murine skin inflammatory responses to contact allergens. However, the role of MCs in chronic recurrent skin inflammation, the clinical picture in patients with allergic contact dermatitis, has not been investigated in detail. Here, we have studied the role of MCs in skin inflammatory responses to repeated exposure to the contact allergen oxazolone using genetically MC-deficient C57BL/6-KitW-sh/W-sh (Sash) mice. Sash mice showed increasingly enhanced skin inflammation upon repeated challenge with oxazolone, as assessed by measuring ear thickness ($P < 0.01$ for the third consecutive challenge). The adaptive transfer of MCs to challenge sites, i.e. the ears, of Sash mice completely repaired this phenotype. Interestingly, enhanced inflammatory skin responses to contact allergen exposure resulted in markedly increased CD44 positive T cell populations in the draining lymph nodes and spleen of Sash mice as compared to wild type mice. These data point to a crucial role of MCs in the prevention and/or down regulation of type IV allergic skin inflammation induced by repeated allergen challenge, possibly by effects on antigen-specific T cell populations.

P145

Induction of immunosuppression by aryl hydrocarbon receptor ligands

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The aryl hydrocarbon receptor (AhR) is a widely expressed ligand activated transcription factor. Its main function is the detoxification of small weight molecular detergents. It was shown that ultraviolet radiation (UVR) can activate the AhR in a ligand-independent fashion. This activation appears to be involved in UVR-induced immunosupppression, since AhR knock-out mice are resistant to UVR-induced immunosuppression. In turn, activation of the AhR by the agonist nonylphenol (NP) inhibited the induction of contact hypersensitivity (CHS) in a similar fashion like UVR. Since UVR induces regulatory T cells (Treg) we asked whether the same applies for NP. Adoptive transfer of T cells obtained from mice which were treated with NP before sensitization rendered the recipient mice unresponsive to the specific hapten. This effect was lost when CD4+CD25+ cells were depleted before transfer. In turn, transfer of suppression was successfully achieved by injection of CD4+CD25+ T cells obtained from NP-treated donors. This indicates that similar to UVR AhR ligands can induced Treg which belong to the CD4+CD25+ subtype and inhibit sensitization in an antigen-specific fashion. The immunosuppressive effect on NP was not observed in AhR knock-out mice indicating that NP acts via triggering the AhR. Since NP is a toxic compound its utilization for the induction of Treg is limited. 6-Formylindolo[3,2-b]carbazole (FICZ), a photoxidative product of tryptophan, has been identified as a physiological ligand for the AhR. Hence, we studied whether FICZ exerts similar immunosuppressive effects. Injection of FICZ inhibited the induction of CHS. T cells obtained from these mice mitigated the induction of CHS in the recipients upon intravenous injection. These data indicate the FICZ exerts similar immunosuppressive features like UVR and thus appears to be a suitable non-toxic compound to induce Treg.

P146

α-Melanocyte stimulating hormone protects from autoimmune encephalomyelitis by generating functional regulatory T cells able to suppress pathogenic Th1 and Th17 cells

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The neuropeptide alpha-melanocyte-stimulating hormone (α-MSH) is a potent immunomodulator that is able to induce immunosuppression and tolerance. In the skin, α-MSH is expressed by keratinocytes and has been shown to generate functional regulatory T cells (Treg) in a mouse model of psoriasis. To investigate whether α-MSH is able to inhibit autoimmunity and inflammation in organs different from the skin, such as the central nervous system (CNS), we used the model of experimental autoimmune encephalomyelitis (EAE), a T cell-mediated inflammatory autoimmune disease resembling human multiple sclerosis. Therefore, C57BL/6 mice were actively immunized by subcutaneous injection of myelin oligodendrocyte glycoprotein (MOG35-55) emulsified in Complete Freund's Adjuvant and systemically treated with α-MSH. Whereas PBS treated control mice showed a significant weight loss and developed severe ascending paralysis starting at day 9–10 after immunization, mice injected with α-MSH appeared to be resistant to actively induced EAE. In support of this, flow cytometry and immunofluorescence staining revealed the absence of pathogenic Th1 as well as Th17 cells from brain tissue of α-MSH treated mice. Moreover, using gene expression assays we detected reduced levels of pro-inflammatory cytokines like TNF-α, IFN-γ or IL-22 in the CNS from α-MSH injected mice versus controls. To assess whether the inhibition of pathogenic effector T cells was mediated by the induction of Treg we characterized the numbers, phenotype, and function of Treg isolated from brain tissue of α-MSH and PBS treated mice at the beginning of hind limb paralysis. Notably, we detected up-regulated numbers of Foxp3+ Treg expressing characteristic markers, such as Helios, CTLA-4, Neuropilin-1, IL-10, and TGF-β in brain tissue from α-MSH treated mice. Of note, these Treg were functional as they efficiently inhibited the proliferation of effector T cells *in vitro*. Since in a mouse model of psoriasis α-MSH has been shown to expand Treg via the induction of tolerogenic dendritic cells (DC) we analyzed the DC phenotype in draining cervical lymph nodes from immunized α-MSH and PBS treated mice. Interestingly, DC from α-MSH injected mice expressed increased levels of PD-L1, PD-L2 or IL-10 and down-regulated typical DC maturation markers like CD80, CD86, and IL-12 pointing to the induction of tolerogenic DC in MOG35-55 immunized and α-MSH treated mice. To elicit its immunomodulatory effects, such as the induction of tolerogenic DC, α-MSH needs to bind to one of 5 known melanocortin receptors whereas mainly the melanocortin-1 receptor (MC-1R) was shown to mediate the impact of α-MSH on immune cells. Thus, we induced EAE in MC-1R deficient mice to decipher the role of this receptor on the beneficial effects of α-MSH on EAE induction and progression. Strikingly, α-MSH treated MC-1R deficient mice developed hind limb paralysis similar to PBS treated controls demonstrating that signaling via a functional MC-1R is essential for the α-MSH mediated prevention of EAE. Together, our data indicate that α-MSH by binding to MC-1R induces tolerogenic DC and expands functional Treg *in vivo*. These Treg suppress pathogenic Th1 and Th17 effector cells during EAE development thus, suggesting α-MSH as potential therapeutic option for the treatment of patients with moderate multiple sclerosis.

P147 (O06)

Cutaneous 4-1BB/4-1BB ligand signaling is involved in the development of a pruritus-like skin disease

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The 4-1BB/4-1BBL (CD137/CD137L) interaction has various effects on immune cells including T cell activation and the regulation of cell viability. The tumor necrosis factor (TNF) family ligand 4-1BBL is expressed on dendritic cells, activated B cells, and macrophages, whereas the receptor 4-1BB is up-regulated upon activation and can be found on effector T cells, regulatory T cells, mast cells, eosinophils, neutrophils, and NK cells suggesting a widespread immunomodulatory function of this signaling pathway. Accordingly, cutaneous antigen-presenting cells express 4-1BBL and 4-1BB is detectable in the skin upon inflammation. To analyze the effects of 4-1BB/4-1BBL signaling on cutaneous immunity *in vivo*, we generated transgenic (tg) mice overexpressing 4-1BB under control of the keratin 14 (K14)-promoter in basal keratinocytes. K14-4-1BB tg mice showed a uniform transgene expression and those founder lines with the highest 4-1BB gene- as well as protein-levels in naive skin were chosen for further analysis. Interestingly, beginning at the age of 3 months K14-4-1BB tg mice developed inflammatory skin lesions at the ears, snouts and neck. To analyze whether cutaneous 4-1BB overexpression resulted in autoimmunity mice were analyzed for the presence of autoantibodies in the serum and moreover, the renal function was assessed. Since we did neither detect anti-nuclear antibodies nor identify immunoglobulin depositions at the kidney tissue, skin lesion development in tg mice was most likely not attributable to systemic autoimmunity. However, immunohistology of lesional skin revealed characteristic hallmarks of human pruritus such as epidermal hyperplasia, irregular acanthosis, fibrosis, collagenosis, and the infiltration of lymphocytes like T cells, mast cells,

and eosinophils into the dermis. In support of this, we observed increased frequencies of scratching in K14-4-1BB tg compared to wildtype mice when the animals were video monitored over 24 h. Notably, in particular mast cell numbers were significantly up-regulated in lesional skin from tg mice as evidenced by immunofluorescence staining using antibodies against CD117 (c-kit) and FcεRI. As mast cells, by releasing IL-31, a cytokine that has been implicated in itch, can contribute to pruritus development we quantified the IL-31 expression and observed elevated IL-31 mRNA levels in lesional skin from K14-4-1BB tg mice compared to controls. Immunophenotyping of inflammatory cells in human prurigo revealed that in particular CD8+ T cells infiltrate cutaneous lesions. Moreover, in inflammatory skin mast cells have been shown to regulate CD8+ effector T cell functions via 4-1BB/4-1BBL signaling. Hence, we quantified the numbers and phenotype of CD8+ T cells in skin lesions and regional lymph nodes from K14-4-1BB tg mice. Interestingly, whereas the numbers of total CD8+ T cells were similar in draining lymph nodes we detected up-regulated levels of proliferating CD8+ T cells expressing activation- and cytotoxic markers as well as pro-inflammatory cytokines in lesional skin from K14-4-1BB tg mice compared to controls. Together, these data suggest a role of 4-1BB/4-1BBL signaling in the development of itch and a pruritus-like skin disease possibly via recruitment of mast cells to lesional skin and activation of CD8+ effector T cells.

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Tolerogenic IL-10-modulated human dendritic cells: induction of anergic regulatory T cells by mature CD83highCCR7highHLA-DRhigh as well as immature CD83lowCCR7negativeHLA-DRlow DC subpopulations

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Our previous studies demonstrated that human IL-10-modulated, tolerogenic dendritic cells (IL-10DC), which are capable to induce anergic regulatory CD4+ T cells (iTregs), consists of two subpopulations, mature CD83highCCR7highHLA-DRhigh and immature CD83lowCCR7negativeHLA-DRlow. Here, we investigated both IL-10DC subsets with regard to their phenotype and tolerogenic capacity to generate anergic iTregs in detail. Initially, we compared the expression of costimulatory and inhibitory molecules of the B7- and ILT-family between human fully mature DC (mDC) and both IL-10DC subpopulations. As compared to mDC, the CD83low IL-10DC subset revealed a significantly diminished expression of costimulatory molecules (CD80, CD86, B7-H2, CD40) accompanied by a slight upregulation of inhibitory molecules (B7-H1, ILT3, ILT4). In contrast, we observed minor changes in expression of costimulatory molecules but significantly increased levels of inhibitory molecules on CD83high IL-10DC, demonstrating significant differences in expression of costimulatory and inhibitory molecules (B7-H1, B7-DC, ILT3) between the two IL-10DC subsets. FACS sorting of both subpopulations was performed with regard to the high or absent expression of CCR7 (representing CD83high or CD83low IL-10DC). Subsequently, coculture experiments with nave CD4+CD45RA+CD25low T cells were conducted. Notably, primary stimulation and restimulation experiments exhibited that both subpopulations, in contrast to mDC and regardless of their maturation state, induced anergic CD4+ T cells as evaluated by a significantly reduced T cell proliferation and diminished Th1 and Th2 responses (reduced expression/production of T-bet, IFN-γ; GATA-3, IL-5, IL-13; IL-2). In addition, we established in preliminary experiments that IL-10DC-induced T cell population exhibited regulatory activities and suppressed the activation of responder T cells. However, in these suppressor assays, iTreg population induced by IL-10DC lost its anergic state, regardless of the stimulation used (syngeneic mDC, anti-CD3+ anti-CD28mAb, CD3negativePBMC + anti-CD3mAb). In conclusion, both phenotypes of IL-10DC subpopulations, mature CD83highCCR7highHLA-DRhigh and immature CD83lowCCR7negativeHLA-DRlow, display properties of tolerogenic human DC which may be used as targets for the development of novel therapeutic approaches for allergies, autoimmune disease or transplant rejections.

P149

Human 6-sulfo LacNAc (slan) dendritic cells have molecular and functional features of an important pro-inflammatory cell type in lupus erythematosus

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Lupus erythematosus (LE) is an autoimmune disease that is considered to be the result of inflammatory dendritic cells driving an IL-23- and IL-17-induced immunopathology. However, the identity of the dendritic cells supporting this immune response is not known. We recently described human 6-sulfo LacNAc-dendritic cells (slanDCs) with a strong Th1- and Th17-T-cell capacity and identified slanDCs as inflammatory dermal dendritic cells in psoriasis locally expressing IL-23 and TNF-α. In this study, we asked for the relevance of slanDCs in LE.

Staining skin sections from LE patients with an anti-slan mAb we identified slanDCs at increased frequency in affected skin lesions of systemic and cutaneous LE. SlanDCs were found scattered within the dermis and clustered in lymph follicle-like structures with T cells in the periphery. Dermal slanDCs are positive for TNF-α and when slanDCs are cultured in the presence of serum from patients with LE they produce TNF-α. Stimulatory components of LE serum were previously identified as autoimmune complexes with ssRNA binding to TLR7 and TLR8. PCR analysis of slanDCs revealed the expression of TLR7 and TLR8. This combined TLR7/8 expression is in contrast to CD1c+ DCs and pDCs that either express TLR8 or TLR7. Accordingly, only slanDCs responded to ssRNA and highly selective TLR7/8-ligands with a strong TNF-α- and IL-12-production. Interestingly, slanDCs did not produce IFN-α. In conclusion, our data provide strong evidence for slanDCs as having molecular and functional features of a pro-inflammatory myeloid DC type involved in the immunopathogenesis of LE.

P150

Disruption of the epithelial barrier favors persistence of pathogens

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Experimental mouse models of bacterial skin infections that have been described show that pathogenic microorganisms can readily invade the epidermis and dermis to produce localized infections. However, these *in vivo* models have the limitation that pathogens need to be injected subcutaneously into the skin of mice, which does not resemble the natural way of skin infection. We describe a novel mouse model for the analysis of cutaneous colonization and persistence of bacteria such as *S. aureus*. We determined how the level of a barrier disruption by tape-stripping correlates with *S. aureus* persistent skin colonization, concomitant induction of cutaneous inflammation and infection. Furthermore, we investigated for the first time how murine skin responds to *S. aureus* colonization in a physiological setting by analyzing proinflammatory cytokines and antimicrobial peptides (AMPs) in mouse skin. We show that the efficiency of skin colonization correlated with the induction level of proinflammatory cytokines and AMPs. Importantly, previous cutaneous damage allows skin inflammation to develop, favors *S. aureus* persistence leading to cutaneous colonization or even infection, suggesting an interdependence of cutaneous bacteria and skin. Our study suggests that skin barrier defects are a prerequisite for *S. aureus* to colonize skin and that prolonged colonization is associated with profound cutaneous inflammation.

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Mast cells establish effective anti-tumor immune defense

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Despite detectable tumor-specific T cells in cancer patients immune defense fails to reject the tumors in most cases. However, in cutaneous melanoma, T cell mediated tumor regression is frequently observed and we identified high numbers of mast cells (MC) especially within the area of tumor regression. We hypothesized that accumulated MC otherwise supporting tumor development and growth may orchestrate cancer defense once activated by the appropriate danger signals. One important receptor for danger signals is Toll-like receptor 4 (TLR4) and *in vitro* TLR4 ligation resulted in potent activation of human and mouse MC. Consequently, we analyzed a murine model of cutaneous melanoma: After 1 week of tumor growth, when MC are already recruited to the melanomas, activation of TLR4 significantly enhanced T cell recruitment to the melanomas and tumor defense in wildtype but not in mast cell deficient sash mice. In this model TLR4 ligands selectively target MC because reconstitution of TLR4−/− mice with wildtype MC but not with TLR4−/− MC established effective tumor defense. Searching for underlying mechanisms we identified a selective marked upregulation and secretion of the chemokine IP-10 (CXCL10) following TLR4 mediated MC activation. To prove the *in vivo* relevance of MC-derived IP-10 for effective tumor defense, MC deficient mice were either reconstituted with wildtype MC or with IP-10−/− MC: Only mice reconstituted with IP-10 secreting MC upregulated recruitment of tumor-specific T cells and established potent anti-tumor immune responses upon TLR4 activation confirming the crucial role of MC and MC derived IP-10. In summary our data demonstrate that activation of MC and MC derived IP-10 can orchestrate effective tumor defense recruiting T cells and initiating tumor rejection. These data are very important for the development of future strategies of cancer immunotherapy and highlight a new role of MC in tumor immunosurveillance.

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The reduction of circulating invariant natural killer T cells in lupus erythematosus patients is associated with enrichment at the site of cutaneous inflammation

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Systemic lupus erythematosus (SLE) is associated with a numerical and functional reduction of peripheral blood (PB) invariant natural killer T (iNKT) cells. Limited information exists on the role of iNKT cells in the pathogenesis of lupus erythematosus.

Here, we investigated the frequency and phenotype of iNKT cells in PB and dermal infiltrates from patients with SLE, subacute-cutaneous lupus (SCLE) and chronic cutaneous lupus (CCLE).

PB was obtained from 23 SLE, 6 SCLE, and 11 CCLE patients, and from 30 healthy controls. iNKT cell frequency and CCR4/CCR6 surface expression were assessed by flow cytometry. The numbers and phenotype of skin infiltrating Vα24+Vβ11+ iNKT cells were investigated by immunofluorescence in lesional biopsies from 20 patients, unaffected skin from three patients, and six healthy individuals.

SLE, SCLE, and CCLE patients displayed significantly lower percentages of circulating CD3+6B11+ iNKT cells compared to healthy controls. While CCR6 expression on iNKT cells was enhanced in active SLE patients with or without cutaneous involvement compared to controls, CCR4 was exclusively increased in SLE patients with active cutaneous lesions. Furthermore, iNKT cells were significantly enriched in lesional skin of SLE and CCLE patients, but not in unaffected skin of lupus patients. The majority of lesional iNKT cells expressed IFN-α.

In summary, we demonstrate that the deficiency in circulating iNKT cells in cutaneous lupus erythematosus is associated with migration of activated iNKT cells to the site of cutaneous inflammation. These data underscore the importance of analyzing iNKT cells not only in PB, but also in the target tissues.

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Langerhans cells preferentially internalize HIV-1 virus-like particles after transcutaneous administration

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The skin is an attractive route for the delivery of vaccines because it is easily accessible and contains immunocompetent cells (e.g. Antigen Presenting Cells (APCs). Skin APCs are able to elicit both, vigorous cellular and humoral immunity. Nanoparticle-based vaccines are novel delivery systems, which have shown the ability to penetrate into barrier-disrupted skin and trigger the activation of APCs. Here, we investigated the use of innovative virus-like particles (VLPs) formed by HIV-1 Gag proteins as carriers for HIV-1 antigens. The penetration of such fluorescently labelled VLPs in human skin explants and their uptake by different skin cell populations has been measured by means of flow cytometry and fluorescence microscopy after isolation of cells from the epidermis and dermis, and magnetic sorting of CD1c-positive APCs. Three different cutaneous administration routes were evaluated: (i) transcutaneous (TC) administration after skin barrier disruption by Cyanoacrylate Skin Surface Stripping (CSSS), (ii) TC administration after pricking with micro-needles, and (iii) intradermal injection (ID). The results show that VLPs were internalized by skin dendritic cells (DCs). The VLPs has been detected not only in CD1a-high Langerhans cells (LCs) of the epidermis, but also in CD1a-high cells isolated from the dermis. This cell population is distinct from CD1a-positive dermal dendritic cells and may represent LCs which have migrated out of the epidermis to the dermis. In TC application after CSSS, VLPs were associated with both epidermal LCs and CD1a-high dermal DCs. Similarly, after cutaneous application of VLPs subsequent to skin barrier disruption by means of micro-needles, VLPs were predominantly detected in CD1a-positive cells of the epidermis (LCs) and CD1a-high cells isolated from the dermis. Furthermore, after complete bypassing of the skin barrier with ID injection, uptake was observed predominantly by CD1a-high dermal DCs, i.e. LCs migrated from the epidermis.

In case of HIV infection, mucosal LCs have been shown to internalize the virus and are suggested be responsible for the virus transport to the lymph nodes. The fact that, in this work, independently on the administration method, VLPs were taken-up predominantly by LCs confirm the role of these APCs in the immune answer to the viral infection and indicate that the targeting of viral antigens to LCs by transcutaneous administration has the potential to be a successful vaccination strategy.

Keywords: Antigen presenting cells, Langerhans cells, HIV-1, transcutaneous vaccination, virus-like particles.

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Generation of an Influenza A based therapeutic human papillomavirus (HPV) vaccine

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Cervical cancer is the second largest cause of cancer deaths in women worldwide. Persistent infection with one of the 15 known high-risk human papilloma virus (HPV) types, in particular HPV16, causes all cervical and a subset of genital, anal and oropharyngeal cancers. The viral oncogenes E6 and E7 are maintained and expressed in infected cells. Although prophylactic vaccines are a major accomplishment to protect against new infections, the high occurrence of HPV in the population suggests for therapeutic vaccines to efficiently diminish viral burden.

Here we show the generation of a live-vector-based therapeutic HPV16 vaccine. For this purpose we used a ΔNS1 influenza system which has the advantages of high immunogenicity, availability of different serotypes for prime and booster immunizations (H1N1, H3N2) and lack of DNA intermediates as important safety feature.

As target immunogen we generated an HPV16 E6-E7 fusion construct with a FLAG-tag (16E6E7FL) and cloned into the ΔNS1 locus. For safety concerns a second fusion of E6 and E7 was generated where we introduced deletions that abrogate their biological function (16E6E7mFL). To enhance protein expression the splice donor site for the fusion was further modified. Following viral infection of Vero cells for 12 h, fusion protein-expression was verified by RT-PCR and Western Blot. Protein expression could only be detected when the proteasome inhibitor MG-132 was present, suggesting rapid degradation of the artificial E6-E7 fusion due to impaired protein folding.

Following infection of C57BL/6 mice with high-titer recombinant virus the immune response was characterized by HPV 16 E6 and E7 peptide ELISA, IFN-g ELISpot and MLR experiments. Immune characterization showed peptide specific IFN-g production of splenocytes of vaccinated mice.

The therapeutic and prophylactic capacity of this HPV vaccine approach was analyzed in an *in vivo* mouse challenge model using HPV16 transformed TC-1 tumour cells. Animals vaccinated and then challenged with TC-1 cells did not develop tumours or showed significantly decreased tumour growth rates as compared to mock and empty influenza vector treated animals. Similar results were obtained in the therapeutic setting, when animals were inoculated subcutaneously with TC-1, primed with H1N1 recombinant virus on the same day and boosted with the recombinant H3N2 serotype 10 days later.

These results suggest a promising new approach for therapeutic vaccines against HPV infection and induced disease.

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Osteopontin, a mediator in imiquimod induced Th17 driven psoriasis-like skin inflammation

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Osteopontin (OPN) isotypes have been demonstrated to have proinflammatory functions in autoimmune diseases. Recently, OPN was implicated in the pathogenesis of psoriasis as OPN is overexpressed in lesional skin and serum of psoriasis patients. The immune regulatory functions of OPN in psoriatic Th1/Th17 driven inflammation are unknown. We investigated the function of OPN in a murine model with OPN deficient mice in which skin application of Imiquimod (IMQ), a TLR-7 ligand, induces a skin and systemic inflammatory response which closely resembles Th17 driven psoriatic inflammation. Wt and OPN−/− mice were treated with IMQ cream daily for 6 days which gradually induces skin inflammation, swelling of skin draining lymph nodes and spleen. Compared to wt mice, OPN deficient mice had a delayed onset in IMQ induced ear swelling. Investigation of skin invading immune cells revealed that IMQ treated OPN deficient mice had attracted less CD3+ lymphocytes, NK-cells and MHC-II+ antigen presenting cells to the skin. In contrast to CD4+ cells which were comparable in wt and OPN−/− skin, CD8+ cells were significantly reduced in OPN−/− skin, resulting in an increased CD4/CD8 ratio. Further, IMQ induced swelling of skin draining lymph nodes was inhibited in the absence of OPN. Paralleling the skin findings, in OPN deficient lymph nodes IMQ mediated expansion of B-cells and concomitant reduction of CD4 cells was inhibited, leading to an increase in the CD4/CD8 ratio. This same pattern was also found in spleen, however, spleen swelling was not affected by OPN deficiency. When analyzing IL-17, IL-4 and IFNγ expression by CD4+ T-cells from spleen and lymph nodes we found that IMQ induced expression of all three cytokines. However, OPN deficiency significantly reduced IL-17 and IL-4 production in CD4+ T cells while the reduction of IFNγ was not significant. In conclusion our data suggests that OPN modulates psoriasis-like skin inflammation through altering lymphocyte distribution in skin and draining lymph nodes and by inducing IL-17 expression of inflammatory T cells.

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Processing of the extracellular matrix component laminin generates peptides with antimicrobial and chemotactic activity

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Laminins are multifunctional glycoproteins within the extracellular matrix (ECM) that play an important role in cellular morphogenesis, tissue repair and wound healing. The heterotrimeric laminins consisting of different, and chains are present in tissues as a part of the basement membrane (BM). The C-terminal laminin G domain-like (LG) modules of several laminin chains can be modified by proteolysis generating LG1-3 and secreted LG4-5 modules. In human skin the laminin 3 and 5 chains are expressed mainly by keratinocytes, thence protecting tissues from pathogens. Invasive pathogens breach the BM and degrade ECM proteins. One of the most frequent pathogens isolated from infections in chronic wounds is *Pseudomonas aeruginosa*. The present work shows for the first time, that *P. aeruginosa* is able to alter the laminin 3 expression in keratinocytes by significant increase of laminin 3 on mRNA and protein level. Moreover, we provide evidence that human keratinocytes and fibroblasts process and secrete biologically active peptides from the LG4-5 module of the laminin 3, 4 and 5 chain *in vitro* and *in vivo*. We show that antibacterial LG4-derived peptides support proliferation of human keratinocytes, essential for epithelial wound healing. Interestingly, we could demonstrate that LG4 peptides are able to activate mononuclear cells and can act as a chemoattractant to direct cells via chemotaxis to the site of inflammation and wounding. Together, these findings reveal that laminins have multifunctional roles in skin host defense and are able to protect skin during wounding and/or infection.

P157 (O10)

GARP has immunoregulatory function on the differentiation process of CD4+ T cell

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CD4+CD25+Foxp3+ T cells (Treg) are critical for the maintenance of peripheral tolerance and prevent the activation of autoaggressive T cells in the context of autoimmune and allergic diseases. GARP (glycoprotein A repetitions predominant) is a molecule expressed on Treg after activation that binds TGF-beta and thus is essentially involved in the mediation of T cell suppression. To investigate the potential role of GARP as a therapeutic agent to treat allergic and autoimmune patients we generated soluble GARP protein (sGARP) and analysed the functional assignment of GARP in the differentiation and activation of human CD4+ T effector cell responses. Here we show that sGARP inhibits proliferation and differentiation of nave and resting T cells into T effector cells. This is associated with the induction of Foxp3 and the specific inhibition of effector cytokine production such as IFN-gamma and IL-2. Importantly, tolerogenic activity of sGARP is Treg cell independent. Functional activity was most pronounced in nave cord-blood-derived CD4+ and resting peripheral CD4+CD45RA+ T cells but not observed in differentiated CD4+CD45RO+ effector T cells. Notably, the Foxp3 induction and cytokine repression by sGARP can be inhibited by blockade of TGF-beta-signalling pathway, suggesting that sGARP function is at least in part dependent on TGF-beta. Repetitive stimulation of nave CD4+ T cells in presence of sGARP directs the induction of iTreg which are able to suppress the activation of T effector cells in coculture. In a pre-clinical humanized mouse model the repetitive application of sGARP efficiently represses graft-versus-host disease (GvHD) including strong inhibition of autoaggressive skin inflammation. These results indicate a crucial role for sGARP in the modulation of peripheral tolerance and open the possibility to use sGARP as an immune modifier in the treatment of allergic diseases and skin-associated autoimmunity.

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Reduced CD18 levels drive Treg conversion into Th17 cells in the CD18hypo PL/J mouse model of psoriasis

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Defective development and function of CD4+CD25high+Foxp3+ regulatory T cells (Tregs) contribute to the pathogenesis of psoriasis and other autoimmune diseases. Little is known about the influence of adhesions molecules on the differentiation of Foxp3+ Tregs into pro-inflammatory Th17 cells, occurring in lesional skin and blood of psoriasis patients. In the CD18hypo PL/J mouse model of psoriasis reduced expression of CD18/2 integrin to 2–16% of wildtype levels is associated with progressive loss of Tregs, impaired cell-cell contact between Tregs and dendritic cells (DCs) as well as Treg dysfunction as reported earlier. In the present investigation, Tregs derived from CD18hypo PL/J mice were analyzed for their propensity to differentiate into IL-17 producing Th17 cells *in vivo* and in *in vitro* Treg-DC co-cultures. Adoptively transferred CD18hypo PL/J Tregs were more inclined towards conversion into IL-17 producing Th17 cells *in vivo* in an inflammatory as well as non-inflammatory environment compared to CD18wt PL/J Tregs. Addition of neutralizing antibody against CD18 to Treg-DC co-cultures *in vitro* promoted conversion of CD18wt PL/J Tregs to Th17 cells in a dose-dependent manner similar to conversion rates of CD18hypo PL/J Tregs. Reduced thymic output of nTregs and peripheral conversion of Tregs into Th17 cells therefore both contribute to the loss of Tregs and the psoriasiform dermatitis observed in CD18hypo PL/J mice mice. Our data overall indicate that CD18 expression levels impact Treg development as well as Treg plasticity and that differentiation of Tregs into IL-17 producing Th17 cells is distinctly facilitated by a subtotal deficiency of CD18.

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Cutaneous RANK-RANKL signaling down-regulates primary anti-bacterial immunity

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Infectious diseases frequently occur in humans and *Staphylococcus aureus* (*S. aureus*) represents the bacterial pathogen causing the majority of cutaneous infections. Bacterial infections are controlled by the innate as well as adaptive immune system and in this context pathogenic microorganisms need to suppress host anti-microbial immune responses. For the suppression of cellular anti-microbial immunity, regulatory T cells (Treg) play a crucial role since Treg are able to inhibit MHC class I- and MHC class II-restricted immunity. Previously, by using transgenic (tg) mice over-expressing RANKL (CD254) in the epidermis (K14 RANKL tg mice) we have demonstrated that RANK-RANKL signaling plays an important role for the peripheral expansion of Treg. Since RANK and RANKL are up-regulated in inflammatory skin lesions we intended to investigate the relevance of RANK-RANKL interactions for the regulation of cutaneous anti-bacterial immunity. Therefore, K14 RANKL tg mice and wildtype (wt) controls were intradermally infected with $2 \times 10e7$ colony forming units (CFU) of the *S. aureus* strain SH1000. Notably, tg mice developed larger skin lesions compared to wt controls, which was paralleled by altered levels of bacteria in lesional skin of tg versus wt mice as evidenced by Gram staining and quantitative real-time PCR. FACS analyses of cell subsets in lymph nodes draining cutaneous lesions revealed up-regulated numbers of Treg in *S. aureus* infected K14-RANKL tg mice compared to wt controls suggesting that the expansion of Treg might result in the inhibition of anti-bacterial immune responses in K14-RANKL tg mice. Thus, we quantified the numbers of effector cells known to play a role in anti-bacterial immunity, such as neutrophils, macrophages, Th1 or Th17 cells in cutaneous lesions and in regional lymph nodes. Interestingly, we could show that the numbers of macrophages and the neutrophil count was reduced in cutaneous lesions from tg compared to wt mice. Moreover, in lymph nodes draining lesional skin from K14 RANKL tg mice we detected decreased levels of CD4+ T cells expressing transcription factors, surface markers as well as pro-inflammatory cytokines specific for Th1 or Th17 cells suggesting that Treg might indeed have suppressed primary anti-bacterial immune responses finally leading to larger skin lesions. To analyze whether RANK-RANKL signaling also affects memory responses wt and K14 RANKL tg mice were re-infected with 2×10^7 CFU of the *S. aureus* strain SH1000 10 weeks after the primary infection. Similar to primary infected mice, re-infected K14-RANKL tg mice developed larger skin lesions, whereas the difference in skin lesion size between wt and tg mice was comparable after the first and second bacterial challenge suggesting insignificant effects of RANK-RANKL signaling on anti-bacterial memory. In support of this, we observed similar numbers of central and effector memory cells in re-infected wt and tg mice as evidenced by CD62L and CCR7 staining indicating a rather minor role of the RANK-RANKL pathway in the regulation of memory anti-bacterial immune responses. Together, these data indicate that RANK-RANKL interactions are critically involved in the suppression of primary innate and adaptive anti-bacterial immunity but most likely do not modulate memory responses.

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The anionic dermcidin-derived antimicrobial peptide DCD-1L acts by ion channel like activity

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Dermcidin (DCD) is a protein, which is constitutively expressed in human eccrine sweat glands. By postsecretory proteolytic processing in sweat the dermcidin protein gives rise to anionic and cationic DCD-peptides with a broad spectrum of antimicrobial activity. We could show previously that the anionic dermcidin-derived peptide DCD-1L inhibit significantly bacterial macromolecular synthesis (RNA, DNA, protein) within the first minutes without binding to microbial DNA or RNA. Further investigations by CD-spectroscopy and conductance measurements with artificial phospholipid membranes showed that DCD-1L is able to form small pores in the bacterial membrane which leads to ion efflux and bacterial death. Atomic force microscopy was used to visualize the morphological changes in the cell envelope upon interaction with DCD-1L. By this a destabilization of the bacterial membrane could be shown after addition of DCD-1L. Furthermore, divalent ions increase antimicrobial activity of DCD-1L. The shorter cationic DCD-derived peptide SSL-25 is also expected to induce ion channels, probably even in synergy with DCD-1L, and will be analysed in future.

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Palmitoylethanolamide: a novel endogenous bio-regulator of human skin and hair follicles

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Palmitolyethanolamide (PEA) is an endogenous lipid, which is a member of the endocannabinoid family. PEA is reported to have anti- inflammatory, nociceptive, neuoroprotective properties. We have previously shown that the endocannabinoid, anandamide (AEA) inhibited human hair follicle (HF) growth, human keratinocytes proliferation and excessive human skin mast cell (MC) maturation and activation. However, the effect of PEA on human skin *in situ* has not been well-explored.

To investigate the effect of PEA on various types of human skin cells, we performed human full thickness skin and microdissected human HF organ cultures with various concentrations of PEA. These organ cultures were performed under serum-free conditions.

Although PEA did not affect hair shaft elongation, it increased the percentage of HFs in catagen by day 6 in organ culture. In line with this observation, the proliferation of hair matrix keratinocytes was inhibited by PEA. These results suggest that PEA has potent hair growth-inhibitory properties by premature termination of the hair cycle growth phase.

PEA did not affect epidermal melanin content as evaluated by Masson Fontana histochemistry in human organ cultured skin. However, in common with AEA, PEA significantly inhibited the proliferation of epidermal keratinocytes *in situ*. Interestingly, this was abrogated by co-administration with CB1 specific antagonist (AM251) and PPAR-α antagonist (GW6471), but not by CB2-specific antagonist (AM630). Furthermore, quantitative MC histomorphometry demonstrated that PEA inhibited human skin MC degranulation induced by an endogenous MC secretagogue, substance P, *in situ*.

Our study suggests that PEA exerts cannabimimetic 'inhibitory tone' on human hair matrix keratinocyte proliferation (thereby inhibiting human HF growth) and human skin MCs degranulation *in situ*. PEA is also shown to inhibit human epidermal keratinocyte proliferation at least in part via CB1 and/or PPAR-α mediated pathway. In addition, our data encourage one to utilize human skin/HF organ cultures as physiologically and clinically relevant model systems for investigating the effects of PEA on different types of cells *in situ*.

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Enhanced expression of antimicrobial peptides in patients with epidermodysplasia verruciformis

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Epidermodysplasia verruciformis (EV) is a rare genetic dermatosis associated with high risk of malignancy. EV patients are particularly susceptible to infections by human papillomaviruses (HPV). The down-regulation of the adaptive immune system causes widespread development of flat warts and pityriasis versicolor-like lesions beginning in early childhood. These life-long persisting HPV-induced lesions show a tendency to evolve with time into malignancies (basal cell carcinomas/BCC, squamous cell carcinomas/SCC) and premalignancies (actinic keratoses/AK, Bowen's disease/MB) preferentially in the sun exposed areas. Surprisingly, individuals with EV are not prone to other viral, bacterial, parasitic or fungal infections. Though the impairment of the adaptive immune system in EV patients is well known, the innate immune system in EV has not been fully characterized yet. Therefore we hypothesized that the observed absence of co-infections in EV patients may be caused by the induction of antimicrobial peptides (AMPs) which are recognised as cutaneous antimicrobial and immunomodulating agents. To address this issue, we investigated the expression of the AMPs human beta defensin (hBD)-2, hBD3, ribonuclease 7 (RNase 7) and S100 A7 (psoriasin) in 37 different tissue samples of EV patients ($n = 4$). These samples included non-tumorous skin, AK, MB, SCC were compared to age- and gender-matched samples of normal skin, AK, MB and SCC of non-EV patients ($n = 96$). Semiquantitative analysis of immunohistochemistry revealed overexpression of psoriasin and hBD-3 but not of hBD-2 and RNase 7 in non-tumorous skin of EV patients in comparison to healthy controls. All four AMPs were upregulated in AK of EV patients. In addition, the expression pattern was different to non-EV-AK demonstrating a more pronounced distribution in the basal and suprabasal epidermal layers. Similar results were obtained for MB with the exception of psoriasin which was equally expressed in EV and non-EV-MB. AMPs were highly expressed in most SCCs but no differences between EV and non-EV patients were observed. The increased expression of AMPs in non-tumorous skin and in premalignancies of EV patients might explain the absence of bacterial superinfections despite the well recognized immune defect. AMPs are also known to promote wound healing. The fact that wounds in EV patients mostly heal rapidly without any complications could also be due to the high expression of AMPs in EV.

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Identification of heterogenous human Treg cells subsets with implications for the pathogenesis of Acne inversa

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Immune responses are tailored to protect against particular types of pathogen encounters. A successful immune defense strategy however also requires intricate negative regulation to restrict inflammation induced host damage. FOXP3+ regulatory T (Treg) cells are a broadly acting and potent anti-inflammatory population of CD4+ T cells essential for maintaining immune homeostasis and preventing autoimmune inflammation. Although Treg cells are generally considered to be a separate lineage of CD4+ T cells, recent murine studies have indicated that they use different transcriptional programs to regulate Th1, Th2, or Th17 responses, and that these are associated with the expression or activation of specific T helper cell-associated transcription factors. This implicates phenotypic and functional heterogeneity within the Treg compartment. We therefore set out to analyze if Treg cells in humans also display functional specialization. We could demonstrate the existence of distinct human Treg subsets with different migration capacities that correlated with different types of immune functions such as cytokine production. These Treg subsets matched their Th1, Th2 and Th17 effector cell counterparts (Tcon), but retained their suppressive function. Treg as compared to Tcon cells showed a homing bias towards the peripheral body surfaces such as the skin where they are expected keep microbiota induced immune responses in check. We could also demonstrate that in inflammatory diseases the relative composition of subsets within the Treg compartment is altered. In Acne inversa, skin homing Tregs are reduced whereas Th17 cells are increased.

Thus, we could demonstrate a so far unrecognized functional heterogeneity within the human Treg compartment and a correlation with alterations in its relative composition with inflammatory diseases such as Acne inversa.

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Induction of immunosuppressive MDSC by extracorporeal photopheresis in patients with chronic GvHD

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Objective: Chronic Graft versus host disease (cGvHD) remains the main non-relapse related cause of death in allogenic hematopoetic stem cell transplantation. In general, treatment of cGvHD is based on administration of immunosuppressive drugs. However, extracorporeal photopheresis (ECP) has become a well-accepted treatment for cGvHD. Induction of regulatory T-cells (Treg) by ECP seems to be important against cGvHD, though the mechanisms of Treg induction still remain enigmic. Recently, CD14+HLA-DRlow monocytic MDSC (moMDSC) were found to support Treg differentiation in humans. Therefore, we hypothesize that ECP induces moMDSC which in turn facilitates Treg differentiation in patients with cGvHD.

Methods: cGvHD was diagnosed according to NIH criteria. Two consecutive cycles of ECP were performed per week for 12 weeks. Blood was obtained after informed consent from patients with cGvHD and from age and gender matched healthy donors (HD). After peripheral blood mononuclear cells (PBMC) isolation by Ficoll, Treg and moMDSC frequencies were determined by flow cytometry. Cells were isolated by magnetic bead based separation for functional analyses. MoMDSC function was tested in autologous CFSE-based-proliferation assays. In addition, the ability moMDSC to drive differentiation of Treg was tested in autologous co-cultures of moMDSC and CD4+ T-cells.

Results: Patients ($n = 4$) with cGvHD showed lower Treg and moMDSC frequencies as compared to healthy donors ($n = 6$) ($P < 0.05$). Serial measurements performed at week 4, 8 and 12 after starting ECP treatment showed a significant increase of moMDSC and Treg frequencies in three out of four patients ($P < 0.05$). *In vitro*, moMDSC inhibited polyclonal T cell proliferation and were found to induce T cells with the phenotype of Treg.

Conclusion: Our preliminary data indicate that ECP enhances the frequency of moMDSC in cGvHD patients. MoMDSC mediate suppression of T cell proliferation and support Treg differentiation *in vitro*. Thus, moMDSC induction by ECP might be of great therapeutic relevance in patients with cGvHD.

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Lymphocyte clonal expansions drive global T cell repertoire bias in generalized pustular psoriasis

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Psoriasis is a T-cell mediated autoimmune skin disease with a heterogeneous genetic background. Clonally expanded, tissue-infiltrating CD8+ T cells are thought be a major effector, and the highest genetic risk is associated with HLA-Cw6. Also, genetic studies indicate important roles of innate immunity in psoriasis etiology. Generalized pustular psoriasis (GPP) is a most severe inflammatory subtype of psoriasis, which may precede or follow onset of psoriasis vulgaris (PV), or can occur independently. Recently, functional mutations in IL36RN, a physiological antagonist of IL-1 signal pathway, have been reported in GPP patients, confirming that innate immunity contributes to GPP onset. In this study, we focus on GPP to elucidate the questions; what causes GPP, psoriasis pustulation?

Seven GPP patients were analyzed for their genetic background and signs of T cell activation. We identified one patient with a homozygous and one with heterozygous IL36RN mutation. Spectratyping of the T cell receptor (TCR) beta chain recombinations showed globally skewed CDR3 fragment lengths in circulating CD8+ T cells of GPP patients compared to healthy controls, indicating that GPP involves oligoclonal T cell activation and expansion. Single cell TCR analysis of blood and skin infiltrating CD8 T cells confirmed multiple T cells with identical TCR rearrangements. Thus, this systemic skewing in CD8+ T cell repertoire was attributed to selective expansion of particular T cell clones in the skin. We also examined how innate immune activation induces antigenic specific lymphocyte activation *in vitro*.

From these data, we conclude that GPP may involve proinflammatory reactivity in the innate arm of the immune system, which promotes an antigen-specific immune response in the skin. Expanded clonal lymphocytes are finally accumulated in biased repertoire of CD8+ T cells at systemic level. This study may provide basic knowledge for GPP onset and/or pustulation of psoriasis, which can be utilized to not only control severe psoriasis activity, but also prevent disease onset in a genetically predisposed individual.

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Beta2 integrin-dependent activation of the NADPH oxidase NOX2 in wound macrophages is essentially required for physiological wound healing

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The beta2 integrin family of adhesion molecules are key regulators of cell-cell interactions in the inflammatory phase of wound healing. Their importance is underlined in patients suffering from Leukocyte Adhesion Deficiency Syndrome type 1 (LAD1). LAD1 patients have mutations in the gene encoding the common beta subunit (CD18) of the beta2 integrins and show spontaneous skin ulcerations and severe wound healing disturbances.

The CD18−/− mouse model, recapitulates the severely impaired wound healing phenotype of LAD1-patients. In this model we previously showed beta2 integrins to control the phagocytosis of apoptotic neutrophils (PMN) by macrophages (Mf) with subsequent release of reactive oxygen species (ROS) and active TGF-beta1 at wound sites, and thus normal wound healing. In Mf, ROS are mainly generated by the NADPH oxidase NOX2.

This project aims to elucidate whether insufficient ROS production due to impaired phagocytic activation of NOX2 in Mf at wound sites drive the reduced release of active TGF-beta1 and impaired wound healing in CD18 deficiency.

In vivo imaging experiments using the redox-sensitive chemiluminiscent substrate L-012 revealed significantly reduced amounts of ROS at wound sites of CD18−/− mice when compared to wildtype (WT) control mice. Remarkably, injection of the oxidative burst inducer Rotenone around wound margins of CD18−/− mice rescued their wound healing defect to WT levels. Further, this effect was completely abolished by co-injecting Rotenone along with the hydrogen peroxide scavenger, Ebselen but not by co-injection with the superoxide dismutase SOD. *In vitro* co-culture experiments of WT Mf

with apoptotic PMN confirmed that co-incubation with Rotenone significantly increased active TGF-beta1 release from Mf. Thus, proper levels of ROS may be essentially required for TGF-beta1 activation by Mf and physiologic wound healing.
Analysis of NOX2 activation in co-cultures of Mf with apoptotic PMN by fluorescence lifetime imaging showed that NOX2 was strongly activated in WT but not in CD18−/− Mf upon phagocytosis of either WT or CD18−/− apoptotic PMN. Most interestingly, p40phox−/− mice lacking functional NOX2 presented with significantly delayed healing of full-thickness wounds when compared to WT mice, very similar with CD18−/− mice. Immunofluorescence staining of WT and p40phox−/− wound sections revealed delayed neo-angiogenesis and myofibroblast differentiation in p40phox−/− mice, while the recruitment of phagocytes to wound sites was not impaired.
Importantly, injection of p40phox−/− Mf around wound margins of CD18−/− deficient mice failed to improve their impaired wound healing, while WT Mf fully restored the wound healing defect in CD18 deficiency. These results may suggest that NOX2 signals downstream of beta2 integrins in Mf and that impaired activation of NOX2 results in insufficient oxidative burst at wound sites and impaired wound healing in CD18−/− mice.
Beta2 integrins and their downstream signaling molecule NOX2 may prove promising targets for modulation of oxidative burst and TGF-beta1 activation by Mf in Mf-dependent inflammatory disorders.

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A novel mouse model to study chemokine receptor CCR2 function and macrophage activation dynamics in skin inflammation

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Repair of damaged tissue requires the coordinated action of inflammatory and tissue specific cells in time and space. Infiltrating cells of the monocyte/macrophage lineage sense a variety of environmental cues of injured tissue and integrate those into a repair response. The molecular determinants that control blood monocyte recruitment to the site of injury as well as the dynamics of macrophage activation and function during healing progression are largely unknown. In this study we addressed two questions: First, what is the specific function of CCR2-signaling for homing of blood monocytes to the site of skin damage? Second, is there a mechanistic link between the dynamics of macrophage activation phenotypes during healing progression and specific repair mechanisms? The role of CCR2 in mediating recruitment of blood monocytes to inflamed tissues has been intensively investigated in various model systems; nevertheless its specific function in this process is still unclear. Interpretation of findings in previously used CCR2-deficient mouse models has been complicated by the fact that reduced macrophage numbers at the lesion might reflect monocytopenia in those models. To examine the functional role of CCR2 in monocyte trafficking a new CCR2-eGFP reporter mouse model as well as mouse models of complete (CCR2D/D) or myeloid cell-restricted (CCR2 fl/flLysMCre) CCR2 gene inactivation were generated. As examined in excision skin wounds in CCR2-eGFP reporter mice, during the entire time course of healing F4/80+CD11b+ cells represented the major fraction of CCR2 expressing cells at the wound site. The accumulation of macrophages at the wound site in CCR2D/D and CCR2 fl/flLysMCre mice was severely impaired, leading to attenuation of tissue vascularization, growth and alphaSMA positive myofibroblasts. Importantly, whereas in unwounded CCR2D/D mice the number of circulating blood monocytes was significantly reduced, in CCR2 fl/flLysMCre mice this number was similar to controls. Thus, our findings in myeloid cell-restricted CCR2-deficient mice provide clear evidence that signaling through CCR2 is critical to direct recruitment of inflammatory monocytes from the blood to sites of tissue damage and propose the CCR2 fl/flLysMCre mouse line as superior model to study functions of CCR2 *in vivo*. Furthermore, as revealed by qRT-PCR gene expression profiling of FACS-sorted wound macrophages, early stage macrophages (F4/80+CD11b+CCR2highLy6Chigh) were characterized by expression of inflammatory and pro-angiogenic mediators (iNOS, IL-1beta, VEGF-A, PlGF), whereas during healing progression late stage wound macrophages (F4/80+CD11b+CCR2lowLy6Clow) revealed a predominantly immunosuppressive phenotype (IL 10, CD206, CD163). Interestingly, at the early stage of repair a subfraction of F4/80+CD11b+ cells combined high levels of iNOS and Arginase-1 expression, which has been reported previously to be a feature of CCR2highLy6Chigh myeloid-derived suppressor cells (MDSCs). Unexpectedly, in skin wounds high expression of iNOS and Arginase-1 was restricted to the Ly6Clow fraction of F4/80+CD11b+CCR2high cells. Therefore, it is unlikely that the monocytic fraction of MDSCs is present during the early repair phase in excision skin wounds. Collectively, our findings provide new mechanistic insights into CCR2-mediated recruitment of blood monocyte subsets into damaged tissue and functional consequences of macrophage plasticity during the sequential repair phases. Our findings might be relevant for novel monocyte-based therapies to promote tissue regeneration.

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Bacterial soft tissue infection in a psoriasis patient: a deficiency of antimicrobial peptides?

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Antimicrobial peptides (AMP) are highly active proteins with a broad-spectrum microbiocidal activity. Human beta defensins (hBD)-2 and 3 were initially isolated from psoriatic scale extracts and other AMP have been identified in high concentrations in psoriatic skin explaining the absence of skin infections in these patients. An unusual case of soft tissue infection under a psoriatic plaque of the elbow was investigated therefore in detail for the expression and secretion of different classes of AMP.
Tissue samples were obtained under local anesthesia from involved psoriatic plaques of the elbow: one with, the other without obvious clinical criteria for a soft tissue infection. As controls, age, sex and localization matched samples were investigated from other untreated psoriasis patients as well as from healthy controls. Immunohistochemical staining was performed with specific antibodies directed against psoriasin (S100 A7), RNase 7 and human beta-defensins (hBD)-2 and -3. In addition standardized skin washing fluids were collected from lesional and non-lesional skin of patients elbows and investigated for the presence of secreted AMP by ELISA. Microbiologic investigations were performed by cultural and molecular (16S rRNA) methods from infected subcutaneous tissue.
Expression of all AMP was remarkably induced in infected and non-infected psoriasis skin of the patient compared to the healthy controls. In skin washing fluids highest concentrations of all AMP were detected in infected psoriatic skin. Microbiologic diagnostic identified Staphylococcus capitis by cultural methods as well as highest homology to Corynebacterium tuberculostearicum and Staphylococcus epidermidis by 16S rRNA analysis.
This extraordinary soft tissue infection in a psoriasis patient may not be caused by a local deficiency of antimicrobial peptides. Other local factors, e.g. a deep penetrating injury bypassing the keratinocyte innate defense system, should be discussed.

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A new task for gamma delta T cells: regulation of hair growth

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Gamma delta T cells regulate epidermal homeostasis, inflammation and wound healing in murine skin. Since wound healing shows multiple cross-connections to the cyclic growth of hair follicles (HFs): e.g. some key factors secreted by gamma delta T cells that promote wound healing are also recognized as major hair growth regulators, and since the number of gamma delta T cells in HFs fluctuates in a hair cycle-dependent manner, we asked whether gamma delta T cells are also involved in hair growth regulation. By quantitative hair cycle histomorphometry, the pattern of depilation-induced HF cycling in back skin was compared between adult wild-type (WT) and gamma delta T-cell-deficient (TCRgd−/−) mice. On days 1 and 8 after anagen induction by depilation, there were no significant differences in the early stages of anagen development between WT and TCRgd−/− mice. On day 17, almost all WT HFs were in anagen VI, while more than 30% of the HFs from TCRgd−/− mice were still in anagen V. Surprisingly, on day 21 after anagen induction when all WT HFs were running through catagen, 20% of the HFs from TCRgd−/− mice had already re-entered into a new anagen cycle. On day 32, TCRgd−/− mice showed a significant acceleration of HF cycling, while the majority of WT HFs were still in telogen. Further, TCRgd−/− mice show decreased skin transcription of IGF1, FGF7, and FGF10 and the number of dermal cells, including mature mast cells, is decreased in TCRgd−/− skin.
These results provide the first evidence that gamma delta T cells execute another important function: regulation of the cyclic growth and regression of HFs. This underscores the intimate connections between wound healing and hair growth, and points to gamma delta T cells as potential novel targets for therapeutic hair growth control.

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Macrophage activation determines collagen fibril architecture

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Recruitment of immune cells is a hallmark of tissue injury and repair. However, full tissue recovery requires resolution of inflammation. The exact mechanisms and dynamics how immune cells interact with tissue resident cells during the subsequent phases of tissue growth and differentiation during the wound healing response are elusive. Here we provide functional evidence that the timely conversion of an early phase pro- into a late phase anti-inflammatory macrophage activation phenotype is critical for effective restoration of tissue integrity following injury (also named M1/M2 conversion). To explore the role of type 2 cytokines for macrophage activation in tissue repair, we generated mice with myeloid cell-restricted Interleukin-4 receptor (IL-4R) alpha deficiency and analyzed the healing response in skin. Quantitative RT-PCR analysis of FACS sorted wound macrophages in myeloid cell-restricted IL-4R alpha deficient mice revealed a disturbed M1/M2 balance in macrophage activation during healing, with a shift towards a prolonged M1 and an attenuated M2 activation. Dysregulated macrophage activation was associated with delayed wound closure and massive hemorrhages in the granulation tissue. A combination of multiple *in vitro* and *in vivo* analysis unraveled an unexpected disturbance of the extracellular matrix architecture in wound tissue of mutant mice, suggestive for impaired mechanical stability. Ultrastructural analysis of wound tissue in mutant mice revealed an abnormal collagen fibril assembly and HPLC-based analysis showed an altered collagen cross-link pattern when compared to control mice. Whereas late granulation tissue in control mice was characterized by a dihydroxy lysinonorleucine (DHLNL) collagen cross-linking pattern which is typical for scar tissue, these crosslinks were significantly reduced in myeloid-cell restricted IL-4R alpha deficient mice. Interestingly, we identified IL-4/IL-13 mediated expression of Found-in-inflammatory-zone-1 (Fizz-1) in macrophages as critical regulator of lysyl hydroxylase-2 (LH-2, Plod-2 gene) expression in fibroblasts. LH-2 is known to play a pivotal role directing the DHLNL collagen cross-link phenotype typically found in fibrosis. Consistently, wound macrophages in myeloid-cell restricted IL-4R alpha deficient mice revealed reduced expression of Fizz-1 and, most interestingly, also expression of Plod-2 was significantly reduced in wound tissue of mutant mice. Collectively, the results of the present study provide new mechanistic insights into myeloid cell-restricted IL-4R alpha signaling giving rise to a macrophage subpopulation in cutaneous repair that through a crosstalk with fibroblasts is critical to develop a functional granulation tissue and matrix architecture important for restoration of stable tissue integrity.

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In vivo imaging of cell proliferation enables the detection of the magnitude of experimental rheumatoid arthritis (RA) by 3′-deoxy-3′-^{18}F-fluorothymidine ([^{18}F]FLT) and small animal Positron Emission Tomography (PET)

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Approximately 1–2% of the world's population suffers from RA, an autoimmune disease that is characterized by systemic and erosive synovitis and joint destruction. Early treatment of RA patients with anti-inflammatory drugs is important for the protection from disease progression and joint destruction. Therefore, the early diagnosis of RA, before the onset of joint destruction, is of special importance.
The aim of our study was to investigate the potential of [18F]FLT-PET, [18F]FLT-PET/computed tomography (CT) and [18F]FLT-PET/magnetic resonance imaging (MRI) to quantify thymidine kinase (tk)-1 activity at different stages in glucose-6-phosphate isomerase (GPI)-induced arthritis and its correlation with Ki-67 protein expression.
We injected nave BALB/c mice intraperitoneally (i.p.) with either GPI-specific antibody-containing serum to induce experimental arthritis or control-serum from healthy C57BL/6 mice. Arthritic or healthy animals were injected with [18F]FLT at days 1, 3, 6 and 8 after the onset of disease and were measured *in vivo* by combined [18F]FLT-PET/CT and [18F]FLT-PET/MRI followed by autoradiography analysis. To verify the *in vivo* PET data, we performed Ki-67 immunohistochemical staining of the ankles and fore paws at the corresponding time points.
Analysis of the different stages of arthritic joint disease revealed enhanced [18F]FLT uptake in arthritic ankles and fore paws compared with healthy ankles or fore paws as early as one day after GPI-serum injection, a time point characterized by clear histological signs of arthritis but without measurable ankle swelling. [18F]FLT uptake in ankles increased at day 3 after the onset of arthritic joint inflammation and reached the maximum observed level at day 8. Ki-67 immunohistochemical staining of the arthritic ankles and fore paws revealed a strong correlation with the *in vivo* [18F]FLT data. Detailed analysis exhibited, that resident cells such as synovial fibroblasts as well as infiltrating cells such as neutrophils stained positive for Ki-67.
Our data clearly prove that [18F]FLT-PET can be used to non-invasively examine inflammation-induced cell proliferation *in vivo* in the GPI-arthritis model, as our measurements were correlated with the ankle thickness, MR- and CT-images, histopathological changes and Ki-67 immunohistochemistry. Combined [18F]FLT-PET/CT and [18F]FLT-PET/MRI measurements enabled us to identify the exact foci of enhanced [18F]FLT uptake in the inflamed ankles and fore paws, to identify the exact anatomic sites of enhanced cell proliferation and to differentiate bone from cartilage and other soft tissues. Thus, non-invasive *in vivo* measurement of cell proliferation using [18F]FLT-PET might be a promising tool to measure the magnitude of inflammation induced cell proliferation in autoimmune diseases such as psoriasis arthritis.

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Low molecular weight fragments of sulfated hyaluronan affect inflammatory macrophage function

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Introduction: Artificial extracellular matrix (aECM) composed of collagen I (coll) and sulfated hyaluronan (sHA) has been identified to exert immunmodulatory properties on inflammatory macrophages (M1). In the presence of aECM, M1 show reduced levels of typically produced inflammatory cytokines, e.g. IL-6, Il-12 and TNF-alpha, in favour of increased levels of the anti-inflammatory/regulatory mediators CD163 and IL-10. Suppression of M1 functions and amplification of immunregulatory macrophages is suggested to be beneficial for fast implant integration and healing of biomaterials. However, it is well documented that high molecular weight (HMW) HA is cleaved into smaller fragments in inflammatory microenvironments. Compared to anti-inflammatory properties of HMW HA, low molecular weight (LMW) HA was described to induce inflammatory immune cell functions. Hypothesizing that post implantation of aECM sHA would also be exposed to its fragmentation e.g. by hyaluronidases and reactive oxide species (ROS), we here test how different sized small fragments of sHA affect M1 functions. Methods: Monocytes were isolated from human peripheral blood and cultured with RPMI-medium supplemented with FCS and GM-CSF to differentiate M1. Sulfated HA fragments (7, 22, 50 kDa) were added after six days and co-cultured for 2–4 days. Production and release of ROS and inflammatory cytokines were assessed by FACS, ELISA and PCR-analyses. Results: We observed that M1 cultured with sHA fragments neither produce ROS nor release TNFα in the presence of sHA fragments alone. By co-stimulation with LPS we detected inflammatory cytokines IL-6 and TNFα at reduced levels and IL-1β and IL-12(p40) at similar levels in M1 cultured with all sHA fragments compared to M1 controls. In contrast, M1 cultured with HMW-HA showed significantly reduced levels of inflammatory cytokines. In accordance with the cytokine profile we detected reduced levels of pNF-kB which regulates inflammatory cytokine production by its activation and nuclear translocation. Conclusion: We can show that sHA fragments of different sizes exert anti-inflammatory effects on M1 functions. Since HA fragments are described to induce inflammatory properties of immune cells, addition of sulfate groups to the HA-disaccharides seems to prevent this immune response. These data emphasize the immunmodulatory effect of sHA and suggest aECM composed of coll and sHA as an effective biomaterial coating.

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Blue light irradiation suppresses dendritic cells activation *in vitro*

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Blue light (DermoDyne®) is a UV-free irradiation suitable for treatment of chronic inflammatory skin diseases, e.g. atopic dermatitis, psoriasis, and hand- and foot eczema. Unlike UV irradiation, we have recently observed *in situ* that epidermal Langerhans cells were not depleted from skin in patients after several treatments. Thus, we have now investigated the effects of blue light on dendritic cells (DC) *in vitro*. DC were generated from buffy coats of healthy donors by pre-selection of monocytes (Mo) and subsequent culture in GM-CSF and IL-4. Immature Mo-DC were harvested on day 6. First, cells were irradiated with a blue light dose of 3.75, 7.5 or 15 J/cm^2. Controls were left untreated, positive controls received LPS/IFNγ stimulation. Interestingly, blue light did not induce maturation as assessed by expression levels of CD80, CD83, CD86 and HLA-DR and had no effect on survival. In addition, irradiation impaired subsequent cell activation and maturation, since lower levels of co-stimulatory molecules were found on irradiated and LPS/IFNγ-stimulated cells as compared to those which received no prior irradiation. However, blue light irradiation did not alter HLA-DR expression. Additionally, irradiated and subsequently stimulated DC secreted lower levels of IL-12p40, TNFalpha, IL-10 and IL-6. Importantly, these results were confirmed using CD19neg CD1c+ primary myeloid DC (μDC) from peripheral blood isolated by MACS separation. Finally, blue light-irradiated or control Mo-DC were co-cultured with CFSE-labelled, allogeneic CD4+ T cells in mixed lymphocyte reactions. T cells stimulated with irradiated DC proliferated significantly less and secreted less IL-2 as compared to those stimulated with untreated control DC. In summary, blue light irradiation of DC *in vitro* suppressed DC activation and their allogeneic stimulatory potential, but did not affect survival. Future experiments will have to investigate the effect of blue light on primary DC from e.g. atopic patients with known alterations together with the mechanism of action in order to better understand how blue light irradiation acts *in vivo*.

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Probiotic lactic acid bacteria show enhanced immune-regulatory capacity in combination with prebiotic oligosaccharides

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The combination of probiotic bacteria with prebiotics (synbiotics) is considered to selectively manipulate the composition of the microbiota in direction to health benefits of the host. This is of special interest regarding the growing application of synbiotics in prevention and treatment of diseases. Aim of the study was to investigate the impact of different bacterial strains in combination with prebiotic oligosaccharides on cytokine release of human monocyte-derived dendritic cells (MoDC). Immature MoDC prepared from peripheral blood of healthy non-atopic volunteers were stimulated with a Lactobacillus and Bifidobacterium strain in different concentrations in the presence of different combinations of prebiotic GOS (galacto oligosaccharides), FOS (fructose oligosaccharides) and AOS (pectin derived acidic oligosaccharides). IL-12p70 and IL-10 were analyzed after 24 h in cell-free supernatants. Incubation of MoDC with different concentrations of the Lactobacillus and Bifidobacterium strains in combination with prebiotic oligosaccharides revealed that GOS/FOS (9:1) and GOS/FOS/AOS (9:1:2) had a significant additive effect on bacteria-induced IL-10 secretion of human MoDC, while the ability of these prebiotic oligosaccharides to increase IL-12p70 production was less pronounced. Enhanced secretion of IL-10 by bacteria-treated human MoDC induced by GOS/FOS and GOS/FOS/AOS suggests immuno-regulatory capacities *in vitro*. Thus, the tested Bifidobacterium and Lactobacillus strains in combination with prebiotic oligosaccharides might be considered as health promoting synbiotics providing new strategies for the therapeutic treatment of diseases including immune-regulatory disorders, such as skin diseases, allergy or infection and open new aspects for the application as allergy preventing ingredients in food.

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The molecular signature of acute cutaneous graft-versus-host disease: a role for IL-22 single-producing T cells?

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Graft-versus-host disease (GvHD) is the major clinical complication of allogeneic hematopoietic stem cell transplantation (HCT) occurring in an acute and chronic form. The question whether similar or different pathomechanisms are operative in acute (aGvHD) and chronic GvHD (cGvHD), remains to be elucidated.

To address this issue, we collected lesional skin biopsies of patients suffering from aGvHD (n = 22) and cGvHD (n = 15) patients. We also obtained serial biopsies of non-lesional skin from HCT recipients at different time points prior and after HCT (n = 14). The cellular infiltrate was assessed by immunofluorescence stainings; interleukins and chemokines were measured by real-time RT-PCR.

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

In aGvHD lesions, there was a predominance of Th2 cytokines (IL-4, IL-13) and Th2 chemokines (CCL17, CCL22). In accordance with these findings, levels of TSLP, a keratinocyte-derived cytokine skewing the immune response towards a Th2 direction, were increased at day 20 after HCT in non-lesional skin of patients who would later develop aGvHD. To our great surprise, levels of IL-22 but not IL-17 were also highly increased in aGvHD but not in cGvHD skin biopsies, suggesting that IL-22 single-producing are major effector cells in aGvHD. The immune response occurring in cGvHD skin lesions was characterized by a Th1 pattern, as evidenced by a relative increase of Th1 chemokines (CCL5, CCR5, CXCL9, CXCL10) as well as Th1 (IFN-γ, IL-12/IL-23p40) and Th17 (IL23p19) cytokines.

Our findings shed new light on the pathomechanisms operative in the different manifestations of cutaneous GvHD and, furthermore, identify molecular signatures to more accurately predict and verify the occurrence of this disease.

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Fungal-mediated induction of the antimicrobial protein RNase 7 in keratinocytes

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Antimicrobial peptides and proteins (AMP) play an important role to protect human skin and other epithelia from infection. The ribonuclease RNase 7 and the S100-protein psoriasin are major skin-derived AMP abundantly expressed on the skin surface. The expression of psoriasin and RNase 7 in keratinocytes can be induced by cytokines and bacteria. However, a potential induction of RNase 7 and psoriasin by fungi has not yet been investigated. To assess the potential capability of human keratinocytes to exhibit a fungal-mediated induction of psoriasin and RNase 7 we treated human primary keratinocytes with different fungal stimuli and analyzed psoriasin and RNase 7 gene and protein expression by real-time PCR and ELISA, respectively.

Treatment of the keratinocytes with the fungal cell wall constituent beta-glucan revealed a slight induction of RNase 7 expression whereas secretion of psoriasin was markedly induced. Similarly, the fungal cell wall preparation zymosan induced the expression of psoriasin whereas RNase 7 expression was only weakly induced.

RNase 7 has been reported to exhibit activity against *C. albicans*. Therefore we stimulated keratinocytes with living *C. albicans* which led to an induction of RNase 7 expression whereas psoriasin expression was not induced. In contrast, culture supernatants of *C. albicans* induced psoriasin expression suggesting that *C. albicans* secretes factor(s) that are able to induce psoriasin expression. This suggests that psoriasin may play a role in controlling the growth of *C. albicans*. Indeed, preliminary experiments revealed that psoriasin is able to reduce the growth of *C. albicans in vitro*.

Since there is some evidence that psoriasin and RNase 7 may play a role in cutaneous defense against dermatophytes we treated keratinocytes with microconidia of the dermatophytes Epidermophyton floccosum and Trichophyton rubrum. Addition of the conidia to the keratinocytes led to a strong outgrowth of fungal hyphae. This was accompanied by a strong induction of RNase 7 whereas psoriasin was less induced. These data indicate that keratinocytes are able to sense the presence of dermatophytes leading to an increased expression of AMP. In line with this, we detected also a Trichophyton rubrum-mediated upregulation of expression of the AMP human beta-defensin-2 and -3. In summary, our data provide further evidence that keratinocytes are able to respond to various fungal stimuli by the differential induction of AMP which may help to control fungal growth.

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The pattern recognition receptor NOD2 is involved in the *Staphylococcus aureus*-induced IL-17C expression in keratinocytes

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The cytokine IL-17C plays an important role in innate immunity due to its capacity to activate the expression of antimicrobial peptides and cytokines. A characteristic of IL-17C is its predominant expression in epithelia including skin. Expression of IL-17C in keratinocytes can be induced by *Staphylococcus aureus*. The aim of this study was to assess the role of the cytosolic pattern recognition receptor nucleotide-binding oligomerization domain protein 2 (NOD2) for the *S. aureus* -mediated induction of IL-17C. An IL-17C luciferase promoter assay in HEK293 cells overexpressing NOD2 revealed that activation of NOD2 leads to induction of IL-17C. This induction was significantly decreased in cells overexpressing the Crohn's disease associated NOD2 mutation 3020insC (1007 fs) or the Crohn's disease and atopic dermatitis associated NOD2-R702W variant. Mutation of three NF-B binding sites in the IL-17C promoter abrogated the NOD2-mediated IL-17C induction indicating that NF-B plays an essential role for IL-17C induction. Infection of primary keratinocytes with living, but not with heat-inactivated *S. aureus* resulted in an induction of NOD2 and IL-17C gene expression. Overexpression of NOD2 in keratinocytes increased the *S. aureus*-mediated IL-17C gene induction whereas overexpression of the NOD2-R702W variant reduced the IL-17C induction by *S. aureus*. Furthermore, siRNA-mediated downregulation of NOD2 in keratinocytes resulted in a decreased induction of IL-17C upon *S. aureus* infection. In summary, our study provides first evidence that *S. aureus* activates NOD2 in keratinocytes resulting in an increased expression of IL-17C, a mechanism which may be dysregulated in atopic dermatitis.

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Staphylococcus aureus activates the inflammasome in keratinocytes leading to increased IL-1beta secretion

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The Gram-positive bacterium *Staphylococcus aureus* is one of the major skin pathogens causing various skin infections. Once keratinocytes get in contact with *S. aureus* they release various cytokines such as TNF-alpha and IL-1beta. The active form of IL-1beta is generated by proteolytical cleavage of the IL-1beta proform. This process can be catalyzed by the inflammasome, a multiprotein complex containing the protease caspase-1. Activation of caspase-1 usually leads to the processing of pro-IL-1beta followed by secretion of biologically active mature IL-1beta. Although it is known that various microorganisms are able to activate the inflammasome in myeloid cells, activation of the inflammasome by bacteria in keratinocytes has not yet been reported. Therefore we studied the influence of the inflammasome on *S. aureus*-mediated IL-1beta secretion in keratinocytes. Treatment

of human primary keratinocytes with living but not with heat-inactivated *S. aureus* induced IL-1beta gene expression as well as release of mature IL-1beta. This release was decreased in the presence of the caspase-1 inhibitor Ac-YVAD-CMK indicating that activation of caspase-1 is required for IL-1beta release. In line with this, *S. aureus* was able to activate caspase-1 in keratinocytes as analyzed by the presence of the p20 caspase-1 subunit which is part of the active caspase-1 enzyme. To further confirm the role of caspase-1 for the *S. aureus*-mediated IL-1beta release we down-regulated the expression of caspase-1 in primary keratinocytes using siRNAs. Primary keratinocytes treated with two different caspase-1 siRNAs showed a significant diminished IL-1beta release upon *S. aureus* stimulation. Similar results were obtained with siRNAs specific for the inflammasome adaptor molecule ASC (apoptosis-associated speck-like protein containing a CARD). These results provide evidence that *S. aureus* induces the secretion of IL-1beta by keratinocytes via activation of the inflammasome. In summary, this is the first report showing that bacteria are able to activate the inflammasome in keratinocytes leading to enhanced IL-1beta release.

P179

Adenosine produced by Treg induces motility and directed migration of denditic cells via adenosine A2A receptor

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In previous studies we have shown that immature dendritic cells (DC) preferentially attach to CD4+CD25+Foxp3+ regulatory T cells (Treg) and not to conventional CD4+ T cells when they are cocultured *in vitro*. This formation of clusters between Treg and DC was dependent on adenosine as blockade of the production of this purine nucleoside in cell cultures inhibited the interaction completely. To identify the source of adenosine in the DC – Treg cocultures we used cells from CD39 KO mice. These cells lack the ectonucleotidase CD39 and are not able to convert ATP into ADP/AMP, which is an essential step on the extracellular pathway to produce adenosine. We generated bone marrow derived DC and isolated Treg from LN and spleen of these mice and cocultured the CD39KO cells together with cells isolated from wt mice. We could detect that CD39KO DC were still able to aggregate with Treg isolated from wt mice, whereas CD39KO Treg did not interact at all with DC generated from wt mice. This impeded interaction, which is due to a lack of adenosine production, was accompanied with a reduced motility and an inhibited directed migration of the DC towards Tregs. Therefore we conclude that the production of adenosine by Treg is essential for guiding DC – Treg aggregation. To identify the responsible adenosine receptor(s) on DC we applied adenosine receptor antagonists' specific for the four different adenosine receptors A1, A2A, A2B and A3. After preincubation of DC with the respective antagonist, Treg were added to the DC and formation of clusters was analyzed by live cell imaging. Here we clearly determined that only the adenosine A2A receptor antagonist blocked the adenosine induced motility of the DC and the formation of clusters with Treg. In contrast, all other adenosine receptor antagonists showed no effects on these events.

As ATP acts as a danger signal in the skin during inflammation and allergic reactions, our data indicate that tissue resident Treg degrade ATP into adenosine, which then mobilizes and attracts immature DC. Treg then interact with the DC in an immunosuppressive manner, preventing excessive immune reactions.

P180

The amino acid sequence EDExxL in the intracellular domain of the DEC205 receptor mediates antigen uptake in dendritic cells by interaction with adaptin-2

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The antigen receptor DEC205 is predominantly expressed on dendritic cells (DC) and mediates efficient endocytosis. Within the intracellular domain of DEC205 a putative targeting sequence (single letter code: EDExxL) was identified, which has high similarity to a sequence (DExxxL) previously shown to target the lipoprotein receptor to lysosomes. To analyze the role of this targeting motive in DEC205 during intracellular routing, we generated fusion receptors containing the IgG-binding extracellular domain of human CD16 and the murine intracellular DEC205 domain (WT-DEC:CD16). We also engineered a receptor containing a mutated EDExxL sequence (EDExxL to AAAxxL: AAA-DEC:CD16) and established stably transfected cell lines of the fibroblasts DCEK. When we analyzed the time course of endocytosis of the ligand human IgG (hIgG) in the two different cell lines we found that WT-DEC:CD16 cells took up the ligand completely within 30 min, whereas AAA-DEC:CD16 showed uptake of only 10% of surface bound hIgG. Comparable results were obtained when the distribution of the DEC:CD16 receptors in steady state, i.e. without incubation with hIgG, was assessed. Here WT-DEC:CD16 receptors were present in prominent vesicles in the cells, whereas AAA-DEC:CD16 receptors remained mostly next to the cell surface. Further analysis of the intracellular routing showed that WT-DEC:CD16 transported hIgG through early endosomes (EEA1+; 10 min after uptake) to late endosomal compartments (LAMP1+; 30 min after uptake). In contrast, DEC:CD16 molecules missing the EDExxL sequence remained in the early endosomes. To analyze the underlying mechanisms we investigated the recruitment of adapter molecules to DEC:CD16 receptors and found colocalisation of adaptin-2 with WT-DEC:CD16 but not with AAA-DEC:CD16. These results could further be supported by co-immunoprecipitation of adaptin-2 with WT-DEC:CD16.

Thus these data indicate that the intracellular targeting of the antigenreceptor DEC205 is initiated by the interaction of adaptin-2 with an EDExxL motive in it's intracellular domain. This interaction is crucial for the effective presentation of antigens by DC and may therefore influence the effective activation of T cells by MHC-peptide complexes.

P181

Triterpenoids from the birch bark show immune modulatory effects *in vitro* and reduce croton oil induced ear swelling *in vivo*

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Pentacyclic triterpenoids like betulin (BE), betulinic acid (BA) and lupeol (LU) are bioactive secondary plant metabolites with various pharmacological effects. Their main *in vitro* effects on immune cells are anti-inflammatory, by modulation of the prostaglandin pathways and inhibition of NF-κB signaling. Nevertheless, there are also few reports about NF-κB activation by pentacyclic triterpenoids and subsequent release of pro-inflammatory mediators. *In vivo* animal studies show edema reduction in chemical induced paw- and ear edema mouse models. Single case studies in human patients show accelerated wound healing by triterpenoids from the birch bark and reduction of exudative inflammatory skin diseases.

In our studies, we examined *in vitro* effects of a triterpene extract (TE) from the birch bark on dendritic cells (DC) and T-lymphocytes. The TE is well characterized with >93% triterpenoids. The main compounds are BE (81.6% w/w), LU (5.9% w/w) and BA (3.1% w/w).

Treatment of LPS activated murine bone marrow-derived dendritic cells (BMDC) with TE resulted in increased upregulation of activation markers (CD80 and CD86) and IL-1β release. Although TE treated DC showed upregulation of positive co-stimulatory molecules, we found anti-proliferative effects on murine T cells by using the mixed lymphocyte reaction (MLR) and α-CD3/α-CD28 stimulation as readout for T cells proliferation. Therefore, other signals than costimulation, which are also required for full T cell activation may be affected by treatment with the TE, resulting in anti-proliferative effects. Experiments with human PBMC derived, *in vitro* generated DC and human T lymphocytes confirmed the mouse data by showing increased activation marker expression on DC and also anti-proliferative effects in the MLR setting.

Based on the anti-inflammatory effects and anti-proliferative effects on murine and human T-cells, TE may be a promising candidate for the treatment of inflammatory skin diseases. Treatment of croton oil induced irritant contact dermatitis with TE resulted in reduced ear swelling. The treatment of T cell mediated inflammatory skin diseases is currently addressed in the mouse model of allergic contact hypersensitivity in the *in vivo* setting. In conclusion, we found immune modulatory effects by TE on murine BMDC and T-cells *in vitro* and reduction of croton oil induced ear swelling *in vivo*.

P182

Cytokine- induced insulin resistance in endothelial cells contributes to the pathogenesis of psoriasis and its co-morbidities

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Psoriasis is a chronic inflammatory skin disease, which not only shows a dermal phenotype, but also has a systemic dimension. This systemic dimension is represented by its co-morbidities such as the metabolic syndrome, diabetes and cardiovascular diseases like atherosclerosis, heart attack or stroke. Due to the mechanistic and histological similarities between a psoriatic and an artherosclerotic plaque, we hypothesize that a common pathomechanism is underlying these formations. A possible link might be represented by inflammation-driven insulin resistance, as under healthy conditions insulin is both cardio protective and anti-inflammatory.

We could previously show that 'psoriatic cytokines' (IL-1β, IL-17, IL-22, IL-23 and TNF-α) can induce insulin resistance in primary dermal microvascular endothelial cells which was measured by the insulin-dependent phosphorylation of PKB.

Moreover we could demonstrate that the c-Jun *N*-terminal kinase (JNK) is the key kinase in mediating insulin resistance by using chemical inhibitors and siRNA mediated knockdown.

In order to investigate the functional consequences of insulin resistance, we examined the expression of adhesion molecules such as E-Selectin and ICAM-1 which are involved in the process of transendothelial migration of lymphocytes. The expression of these molecules on the cell surface is repressed by insulin and altered under conditions of insulin resistance. We could show that these effects do not depend on transcriptional or translational processes, but seem to depend on transport mechanisms. Furthermore we investigated the rolling and adhesion of lymphocytes on the endothelial cell layer under static and flow conditions. We could show that both processes are altered under insulin-resistant conditions, which supports the inflammation in the skin and the formation of artherosclerotic plaques at the endothelium.

Therefore we suggest that under conditions of systemic inflammation as in psoriasis the disturbed insulin response contributes to the pathogenesis of psoriasis and its co-morbidities by regulating the presentation of adhesion molecules on the endothelial surface and thereby contributing to lymphocyte extravasation. Therapeutic approaches interfering with the altered insulin response in psoriasis might be very effective by targeting both the dermal as well as the cardiovascular dimension of psoriasis.

P183

Effects of vitamin D3-induced TSLP overexpression in epidermis of flaky tail mice

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Loss-of-function mutations in the gene encoding filaggrin are a major predisposing factor for the development of atopic dermatitis (AD). We hypothesized that impaired skin barrier function and upregulated expression of epidermal thymic stromal lymphopoietin (TSLP) synergize in the pathogenesis of AD. Thus, adult flaky tail mice with filaggrin deficiency (ft/ft) were topically treated with vitamin D3 (VitD3) on ear skin once daily for 10 days.

Monitoring of ear thickness throughout the VitD3 treatment and histological analysis of ear skin sections on day 10 revealed pronounced ear swelling and slightly aggravated skin inflammation in VitD3-treated mice, when compared to vehicle-treated controls. Furthermore, skin expression of TSLP, IL-13, IL-10 and thymic- and activation-regulated chemokine (TARC/CCL17) was increased upon VitD3 treatment. Although topical VitD3 induces a local AD-like skin inflammation in flaky tail mice, migration of skin-derived dendritic cells (DC) to the skin draining lymph nodes (sdLN) was not increased and serum IgE levels remained unchanged. In addition, numbers of CD4+ T cells, including activated CD4+ T cells and regulatory T cells, in sdLNs were unchanged in flaky tail mice following VitD3-treatment.

Thus, differing from wild-type mice, which develop local and systemic AD-like symptoms after VitD3-treatment, ft/ft mice exhibit an inflammatory response restricted to the skin.

P184 (O01)

Inactivated *Chlamydia trachomatis* coupled to a nanoparticle-based TLR7 agonist induces a protective mucosal CD4+ T cell response

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The genital mucosa is a major site of entry and transmission for pathogens that cause sexually transmitted infections. No mucosal vaccines are available that induce a local cellular immune response providing effective protection against such pathogens. The obligate intracellular pathogen *Chlamydia trachomatis* (Ct) is the most commonly reported sexually transmitted bacterial infection and the world's leading cause of infectious blindness and female infertility.

We designed a vaccine consisting of inactivated Ct with a TLR7 agonist attached to the microbial surface via nanoparticles (T7AaNPs) and, using a well-established mouse model of Chlamydia genital tract infection, we tested its ability to induce protective immunity. Genital and intranasal, but not subcutaneous, immunization with the construct resulted in protection when mice were genitally challenged with infectious Ct up to 6 months after immunization. Interestingly, inactivated Chlamydia alone or mixed together with TLR7 agonists that were not encapsulated in nanoparticles lead to higher Chlamydia loads upon subsequent challenge.

To test which effector cells were mediating protection we immunized DHLMP2a−/− (antibody-deficient), μMt (B cell-deficient), CD8−/−, MHC class II−/− and RAG-2−/− mice and challenged the mice 1 month later in the genital tract with Ct. Protection after immunization with T7AaNPs bound to inactivated chlamydia was observed in the antibody-, B cell-deficient and CD8-deficient mice, while MHC class II−/− and RAG-2−/− mice were not protected against Chlamydia challenge. As might be expected from these data, we were able to transfer to nave mice protection against genital Ct challenge by injecting purified CD4+ T cells from immunized mice. Of note, transfer of CD4+ T cells from mice that had been conditioned with inactivated C. trachomatis alone, conferred enhanced susceptibility to C. trachomatis challenge, indicating that tolerance also depends on CD4+ T cells.

We also tested nave mice that had received CD4+ T cells from transgenic T cells expressing a TCR specific for Ct. These T cells responded similarly to our experimental vaccine and infectious Ct as determined by rate of T cell proliferation and secretion of IFN-gamma, IL-2- and TNF-alpha.
Vaccinating against intracellular bacteria where an efficient CD4+ T cell immune response is required is an especially challenging aspect of vaccine design. With the new technologies described here, we were able to use inactivated Chlamydia linked to a TLR7 ligand to prevent a tolerogenic response and instead induce a potent immunogenic effect. In addition we observed transmucosal protection as we could specifically induce CD4+ memory cells within the genital mucosa by intranasal immunization. This is essential for translating our vaccine against Chlamydia and possibly other sexually transmitted infections to humans.

P185

Host defence to *Staphylococcus aureus* induced skin infection

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Staphylococcus aureus (*S. aureus*) is one of the major human pathogen causing several bacterial skin infections, but also often found on normal skin and in ulcers or chronic wounds. It is not known when *S. aureus* remains a harmless dweller in wounds and when it becomes the cause of soft tissue infection. We hypothesized that these different courses depend not only on its virulence factors, but also on genetically determined resistance of the host.
To study the contribution of host factors to the etiology of soft tissue infection we inoculated different inbred mouse strains subcutaneously with *S. aureus* isolates. We found that higher susceptibility of C57BL/6 mice to *S. aureus* infection correlated with significantly higher foot swelling, increased dissemination of bacteria into inguinal lymph nodes and kidneys as well as lower influx of polymorphonuclear leukocytes than in resistant BALB/c mice.
There is growing evidence that the microenvironment of the infected tissue influences the direction of immune response. To explore the role of the skin as the primary site of infection in regulating immune response, we performed comparative gene expression analysis using microarray technology, RT-PCR, protein expression study and functional clustering early after infection in the skin of resistant BALB/c and susceptible C57BL/6 mice. The number of regulated genes was higher in resistant than in susceptible mice. Accordingly, we found that genes involved in cytokines (IL-1beta, IL-6, S100A8/A9) and chemotaxis (CXCL1, CXCL2, CCL3) were significantly differentially regulated between both mouse strains. The chemokine CXCL2 was strongly up-regulated in resistant BALB/c mice and therefore we studied the effect of CXCL2 in resistant and susceptible mice. Local injection of CXCL2 in resistant mice at the first day of infection resulted in a decreased foot swelling in contrast injection of CXCL2 in susceptible mice resulted in enhanced swelling reaction.
In summary, our study shows a global view of gene expression pattern and revealed a higher expression of immune modulatory molecules in resistant BALB/c than in susceptible C57BL/6 mice. Thus, we revealed genetically determined differences of the epithelial barrier are decisive for the host-specific immune responses of *S. aureus* skin infection.

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CD16a-dependent recruitment of slanDCs and NK cells to immune complexes at the vascular interface

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The deposition of immune complexes (ICs) within tissues or in the vascular bed is a critical pathogenic event in the promotion of autoimmunity-driven inflammatory responses. Fc receptor-mediated interaction of ICs with immune cells contributes to IC-triggered inflammation and tissue damage. We have previously described a highly proinflammatory subset of myeloid CD1c- CD11c+ DCs, identifiable by selective expression of the carbohydrate modification 6-sulfo LacNAc (slanDCs). A hallmark of slanDCs is their high level expression of the low affinity Fc gamma receptor IIIA (CD16a) - an IC receptor associated with ITAM-bearing activating gamma chains. In the present study we used small soluble ICs and human IgG subtypes and applied a flow chamber adhesion assay and time-lapse video microscopy to measure the arrest function of blood leukocytes to immobilized ICs/IgGs under conditions of physiological shear stress. We observed arrest of slanDCs in the presence of shear stress reaching up to 2 dyn/cm corresponding to normal venous blood flow. Other human blood DCs (plasmacytoid DCs and CD1c+ DCs) or T cells completely failed to adhere to immobilized ICs. By selectively blocking the Fc gamma receptors on slanDCs, we clearly show that their shear-resistant adherence to immobilized ICs critically depends on CD16a, while CD32 (Fc gamma RII) expression is irrelevant. In accordance with this, mature slanDCs that have proteolytically downregulated CD16a failed to attach. Using immobilized human IgG subtypes we observed that only IgG3 was able to mediate the arrest of slanDCs. In this setting, too, the arrest of slanDCs to human IgG3 was largely dependent on CD16a. The existence of this CD16a/IC-mediated recruitment at the endothelial interface is supported by data showing enhanced arrest of slanDCs on monolayers of human dermal microvascular endothelial cells that were pre-incubated with anti-endothelial cell IgG antibodies. Finally, slanDCs were frequently found by immunohistochemistry in early skin lesions of allergic vasculitis. Of note, we observed that the subset of CD16a positive NK cells can also be captured by these immobilized ICs, extending this phenomenon to two important immune cell types expressing high amounts of CD16a. Taken together, we have shown that CD16a mediates an efficient shear-stress-resistant adhesion of circulating slanDCs and NK cells to immobilized ICs. These data provide evidence for a novel conduit of rapid FcR-dependent recruitment of immune cells in IC-mediated tissue inflammation.

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Chronic but not acute epidermal barrier disruption triggers mast cells in skin and serum IgE

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Atopic dermatitis (AD) is a common inflammatory skin disease characterized by impaired epidermal barrier function and cutaneous inflammation usually with onset in early infancy. Although the pathogenesis of AD remains ill-defined, the central and initial role of cutaneous barrier dysfunction resulting from mutations in the gene encoding for filaggrin is becoming a paradigm. The purpose of this work was to investigate the ability of chronic versus acute epidermal barrier impairment to trigger AD-like symptoms. For acute barrier disruption, mice were tape stripped (six tapes) once a week for 2 weeks and analyzed 1 week after the last tape stripping. Chronic epidermal barrier disruption was induced by mild tape stripping twice a week for 7 weeks. Acute epidermal barrier impairment induced mild epidermal hyperplasia but no inflammatory dermal infiltrates. The regulatory cytokine IL-10 was increased in tape stripped skin whereas TSLP, IL-13, IL-1β, IL-5 were unchanged. Systemically, plasma IgE levels, numbers of T-lymphocytes or dendritic cells in skin draining lymph nodes remained unchanged. In contrast, chronic epidermal barrier disruption induced epidermal hyperplasia associated with mild inflammatory dermal infiltrates mostly consisting of mast cells. The expression of the inflammatory cytokines TSLP, IL-1β, IL-31 was up-regulated while expression of IL-10 was decreased. Systemically, plasma IgE levels were markedly increased whereas the numbers of T-lymphocytes and dendritic cells in skin draining lymph nodes remained unchanged. Thus, while acute epidermal barrier disruption is followed by a rapid return to steady state conditions, chronic epidermal barrier disruption triggers cutaneous inflammation with features of increased serum IgE.

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A second-generation human papillomavirus RG1-VLP vaccine induces broad-spectrum protection against mucosal and cutaneous types *in vitro* and *in vivo*

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Licensed human papillomavirus (HPV) vaccines are based on virus-like particles (VLP), self-assembled from major capsid protein L1. Both vaccines induce high-titer, yet largely type-restricted antibodies against mucosal high-risk HPV16 and 18, which cause the majority of cervical and anal carcinomas and a subset of other genital and oro-pharyngeal cancers. The quadrivalent vaccine also includes VLP of the mucosal low-risk HPV6 and 11, which induce 90% of genital warts. However, these vaccines may not protect against one third of cervical cancers, and do not target over 20 additional oncogenic mucosal or cutaneous HPV, respectively.
In contrast, immunizations with peptides of minor capsid protein L2 induce low-titer but cross-protective antibodies. Although L2 contains type-common motives crucially involved in early viral infection, L2 is immunologically subdominant in the context of native virions or co-assembled L1 + L1 VLP.
To improve immunogenicity of L2 we have generated chimeric RG1-VLP by genetic insertion of a broadly cross-neutralizing L2 epitope RG1 within the immunogenic DE-surface loop of HPV16L1, resulting in a 360-fold display of RG1 by the assembled particle.
Immunization with RG1-VLP using human applicable adjuvant alum-MPL induced a strong immune response against HPV16 and broadly cross-neutralizing antibodies against mucosal high-risk HPV18/31/45/52/58/26/33/35/39/68/59/68/73/69/53/34, mucosal low-risk HPV6/11/32/40/44/70, common cutaneous HPV2/27/3/76, genus-beta HPV5, but not HPV1/4 in both pseudovirion neutralization assays, and newly established infectivity assays using native cutaneous HPV virions isolated from patients' skin. Using a mouse genital challenge model, passive transfer with RG1-VLP immune serum efficiently protected mice against experimental infection with pseudovirions of high-risk HPV16, 18, 45, 31, 33, 52, 58, 35, 39, 51, 59, 68, 56, 73, 26, 53, 66, 34 and low-risk HPV6, 43, 44, as opposed to mainly type-restricted protection by antisera to homologous L1-VLP. Long-term monitoring of antibody levels and stable cross-protection indicated efficient B-cell memory response to RG1-VLP vaccination.
RG1-VLP is a promising candidate do develop a second-generation single-antigen vaccine against mucosal high-risk, mucosal low-risk and cutaneous HPV.

P189

Activation of inflammasomes by tyrosine complexes

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Inflammasomes are cytosolic protein complexes of innate immune cells that sense various danger-associated molecular patterns (DAMPs). Activation of inflammasomes results in autocatalytic cleavage of caspase-1 which subsequently leads to the conversion of pro-IL-1 beta into its active form. The NLRP3 inflammasome has been demonstrated to be a crucial element in the adjuvanticity of vaccines containing aluminium-based adjuvants. However, the use of alum has raised concerns since it is has been demonstrated that alum has the potential to induce serious neurological and immunological disorders. A different compound that is used as a constituent of allergy vaccination products is the natural amino acid tyrosine. Activation of inflammasomes could therefore also be the mechanism of action of tyrosine complexes, thereby contributing to effective immunization. The human monocytic cell line THP-1 was stimulated with tyrosine complexes (30 ng/ml–300 g/ml) after differentiation with PMA (300 g/ml) and priming with ultrapure LPS (100 ng/ml) to induce transcription of pro-IL-1 beta. Aluminium hydroxide (alum, 200 g/ml) served as a positive control. Stimulation with 300 and 30 g/ml of tyrosine complexes resulted in a significantly higher IL-1 beta secretion compared to the LPS control. Also primary blood monocytes and dendritic cells secreted IL-1 beta upon stimulation with tyrosine complexes. The addition of a caspase-1 inhibitor (YVAD-CMK) prior to LPS stimulation resulted in a reduced IL-1 beta secretion which indicates involvement of inflammasomes in this process. Furthermore, stimulation with tyrosine did not cause additional cell death as alum does. We conclude that tyrosine comlexes are able to induce IL-1 beta, likely in an NLRP3 inflammasome-dependent manner and thereby, tyrosine could be a safer alternative to commonly used alum-based adjuvants.

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Similarities and differences in the pathogenesis of pityriasis rubra pilaris and psoriasis

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Pityriasis rubra pilaris (PRP) is a rare chronic skin disease of unknown etiology and pathogenesis characterized by reddish orange scaly plaques, palmoplantar keratoderma, and keratotic follicular papules. PRP is often mistaken for psoriasis and thereby respective patients receive inappropriate therapy. The aim of our work was to illuminate the pathogenesis of PRP by analyzing the levels of immune mediators in PRP skin lesions and comparing these with the respective levels found in psoriasis lesions. In doing so we intended to learn more about PRP pathogenesis and find molecular markers that would facilitate differentiation between PRP and psoriasis. We demonstrated for the first time a strong expression of many different inflammatory cytokines (e.g. IL-20) in PRP lesions suggesting that PRP is an immune-mediated disorder. Interestingly, the lesional expression of IL-12 was even higher in the majority of PRP cases than in psoriasis lesions, whereas the expression of IL-17A, and IL-22 was mostly very low in PRP compared to psoriasis lesions. These results suggested that in contrast to psoriasis with dominating Th17 and Th22 pathways, in PRP the Th1 pathway prevails. However, the analyses of larger PRP patient collective (10-15) are necessary to exactly describe all of the immunological subtypes of PRP. The low level of IL-22 in PRP lesions may be responsible for the distinct epidermis alterations characterizing PRP and psoriasis. The identified dominance of the Th1 pathway in PRP lesions gave rise to the idea of treating patients suffering from PRP with ustekinumab, a monoclonal antibody against a part of IL-12 and IL-23. Interestingly, the treatment of three patients with ustekinumab affected decisively by PRP resulted in considerable improvement of the disease. Taken together, our study suggests that (i) PRP is an immune-mediated disease, (ii) measurement of cytokines can help in diagnosing PRP, and (iii) ustekinumab treatment is an effective therapy for PRP patients with a dominant Th1 or Th17 pathway.

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B cell development in CD18hypo PL/J mice – caught between CD18 deficiency and autoimmune inflammation

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Psoriasis is a chronic disease affecting the skin in 2-3% of the general population. We previously showed that the CD18hypo PL/J mouse with reduced expression of the common chain of beta2 integrins (CD11/CD18) spontaneously develops a skin disease that closely resembles human psoriasis, with accumulation of activated CD4+ T cells in lesions, impaired regulatory T cell function and activated macrophages contributing to the development of the disease. The possible involvement of B cells in the pathogenesis of Psoriasis, however, is still unclear.

Recently, it has been shown that autoimmune inflammation impairs B cell development in the bone marrow. Additionally, it is known that loss of CD18, which is involved in lymphocyte trafficking, severely affects the distribution of lymphocyte subsets in the periphery.

Therefore, we analysed B cell development and distribution in healthy and diseased CD18hypo, CD18wt and CD18−/− PL/J mice in order to analyse the effect of inflammation and/or CD18 deficiency on the B cell department.

We found that CD18 deficiency led to a dose dependent reduction of B220+ B cells in the bone marrow of PL/J mice. This turned out to be mainly due to reduced numbers of precursor B cells (B220lowIgM-) and immature B cells (B220lowIgMlow). Inflammation in the CD18hypo PL/J mice decreased precursor and immature B cells further.

Within the precursor subset, CD18 deficiency led dose dependently to reduced numbers of pro (CD24- CD43+), late pro (CD24+ CD43+) and pre B cells (CD24high CD43-). The general distribution of the precursor subsets was not influenced by CD18 expression; inflammation, however, changed the distribution due to loss of pre B cells via apoptosis.

Neither CD18 deficiency nor inflammation had an impact on B cell numbers in blood. Total loss of CD18, however, caused increased B cell numbers in spleen, most likely due to impaired emigration of mature B cells. Inflammation in CD18hypo PL/J mice had no impact on splenic B cell numbers.

Analysis of skin-draining lymph nodes showed an increase of total cells in inflamed CD18hypo animals. Complete lack of CD18, however, reduced cell numbers significantly compared to CD18wt mice. Interestingly, this is not mirrored in B cell numbers, as CD18−/− mice as well as diseased CD18hypo mice displayed increased B cell numbers when compared to CD18wt, whereas healthy CD18hypo animals had less B cells than CD18wt animals. These differences arose most likely out of a combination of impaired infiltration (healthy CD18hypo) and increased proliferation (diseased CD18hypo and CD18−/−).

Our results show that inflammatory conditions in the CD18hypo PL/J Psoriasis model impaired B cell development in bone marrow. Additionally, CD18 deficiency obstructed normal distribution of mature B cells, with accumulation in spleen and lymph node while there was no change of mature B cell numbers in blood and bone marrow. It remains to be seen how these changes influence the development of the psoriatic phenotype in the CD18hypo PL/J mouse model.

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Glucocorticoids induce an antiinflammatory murine monocyte which modifies innate and adaptive immune responses

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Monocytes represent a central part of innate immunity. They can differentiate into macrophages and dendritic cells and are crucial for many steps of an immune reaction including clearance of infectious agents as well as active resolution of inflammation.

We recently showed that glucocorticoids induce a specific monocyte phenotype with antiinflammatory properties in humans. These glucocorticoid stimulated monocytes (GCsMs) display increased chemotaxis, produce antiinflammatory mediators like IL10 and show higher capacity for phagocytosis of latex beads, bacteria and Leishmania (L.) major parasites. Moreover GCsMs also exhibit enhanced killing of phagocytized L. major parasites and bacteria despite reduced generation of reactive oxygene species in general. Thus, they could be important for resolution of inflammation and even infections *in-vivo*.

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

We revealed that glucocorticoid treatment of murine monocytes results in a similar antiinflammatory phenotype including functional features like low adhesiveness, but high migratory capacity to chemotactic factors like C5a and leishmania chemotactic factor *in-vitro*. The GC-induced functional phenotype was stable for at least 96 h after stimulation as shown by phenotypic and functional analysis. Moreover GCcMs also interact with the adaptive immune system as they inhibited T-cell proliferation *in vitro*.

Using monocytes tagged with the near-infrared emitting dye DiR and *in-vivo* imaging, we were able to demonstrate that GCsMs have a higher migratory capacity in different inflammatory model systems *in-vivo*. Injection of GCsMs was also associated with faster resolution of inflammation and induction of regulatory T-cells in experimental transfer colitis.

Taken together, GCsMs have the capacity to modulate innate and adaptive immune responses. Since glucocorticoids are physiologically released in inflammatory conditions, they may not only be of pharmacological but also of physiological relevance for active resolution of inflammation and induction of peripheral tolerance.

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Regulation of GM-CSF production by human T helper cells

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GM-CSF is a hematopoietic growth factor with pleiotropic functions. Previous studies on experimental autoimmune encephalomyelitis (EAE) have demonstrated an essential pathogenic role for T cell derived GM-CSF in autoimmunity. However, the role of GM-CSF in human inflammatory diseases has not been established yet nor has its regulation in human T helper cell subsets been addressed. In this study we report the existence of GM-CSF producing human T helper cells *in vivo* that lack co-expression of other lineage defining cytokines such as IFN-γ, IL-4 and IL-17 and their respective transcription factors T-bet, GATA-3 and ROR-γt. Although this suggests the existence of a novel T helper cell subset we could demonstrate that the classical Th1, Th2 and Th17 cell subsets could acquire GM-CSF production abilities upon autocrine IL-2 production as well. In contrast to findings made in mice, human GM-CSF production was not induced in nave T cells nor enhanced on the memory level by IL-1β or IL-23, two cytokines implicated in rendering auto-reactive Th17 cells pathogenic, but instead by IL-2. Therefore GM-CSF production was not restricted to a separate T cell subset but also a universal feature of other polarized human T helper cell subsets. To address its role in autoimmune pathogenesis, we isolated T helper cells from psoriasis plaques and healthy skin and compared their cytokine profiles. T cells from diseased skin had higher expression of IL-17 and IL-22, two cytokines reported in the pathogenesis of psoriasis, but lower levels of GM-CSF than T cells in healthy skin.

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

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Breakdown of extracellular matrix components – a crucial pre-requisite for contact sensitizer induced inflammation

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One of the crucial events for the sensitization to contact allergens is triggering of innate immune responses. Without the induction of a pro-inflammatory cytokine milieu in the skin that facilitates full dendritic cell (DC) maturation, DCs are unable to trigger the activation of nave T cells. Therefore, it is necessary to understand the underlying mechanisms leading to the induction of innate immune responses mediated by contact sensitizers, i.e. to elucidate the factors responsible for the functionality of the non-antigenic component of contact sensitizers. On the one hand this knowledge should help to develop better *in vitro* assays, allowing for the differentiation between contact sensitizers and irritants, and on the other hand aid the development of new causative treatments for allergic contact dermatitis. The replacement of the current treatment with corticosteroids and non steroidal anti-inflammatory drugs is essential due to the high risk of unwanted side-effects associated with the current symptomatic treatment.

To this end, we aimed at the identification of possible endogenous danger signals that might be released after treatment of the skin with contact sensitizers as it is well known that activation of pattern recognition receptors like Toll-like receptors (TLR) 2 and 4 is able to trigger inflammation. We have been able to show that breakdown of high molecular weight hyaluronic acid (HA) into low molecular weight fragments is able to facilitate inflammation in the skin, presumably via activation of TLR2/4 signaling. This breakdown of HA is mediated both by contact sensitizer induced production of reactive oxygen species (ROS) as well as by an up-regulation of the activity of hyaluronidases (HA degrading enzymes). When blocking either ROS production by topical application of antioxidants or by inhibition of hyaluronidase activity we can abrogate contact hypersensitivity (CHS) responses. Interestingly, the inhibition of ROS production prevents both sensitization as well as the elicitation of CHS responses. This indicates that even in already sensitized mice - mimicking the clinical situation in humans - CHS responses can be blocked by prevention of inflammatory responses. In addition, we have now identified a second component of the extracellular matrix (ECM). First experiments indicate that expression of this ECM component is essential for sensitization by contact sensitizers.

Taken together, we show here that for sensitization the generation of ROS and the breakdown of ECM components, i.e. the generation of endogenous damage associated molecular patterns (DAMPs), is a necessary pre-requisite. Thus, by broadening the knowledge about the mechanisms involved in CHS, we point out future strategies for causative therapies of allergic contact dermatitis by targeting innate immune and stress responses.

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TREX1-deficient mice develop autoimmune disease with cutaneous involvement

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Systemic lupus erythematosus (SLE) is a prototypic multifactorial autoimmune disease with prominent cutaneous involvement that is characterized by autoantibody formation against self-antigens and an increased type I interferon (IFN) production in genetically susceptible individuals.

Heterozygous mutations in the most prevalent intracellular 3'-5'- DNA exonuclease TREX1 are associated with the development of systemic lupus erythematosus and can cause monogenic familial chilblain lupus. Chilblain lupus lesions and signs of systemic autoimmunity like autoantibody formation and interferon upregulation are also features of the rare autosomal recessive encephalopathy Aicardi Goutieres syndrome that can be induced by biallelic mutations in TREX1.

The pathogenic link between TREX1 deficiency and autoimmunity has mainly been elucidated by studying TREX1 deficient mice. These animals exhibit dramatically reduced postnatal survival and die of type I interferon-mediated, T and B cell dependent myocarditis. Further signs of autoimmunity in these mice are autoantibody formation and inflammatory organ infiltration.

To investigate a possible link between cutaneous lupus lesions in humans with TREX1 mutations and mice, we closer analyzed the cutaneous phenotype of TREX1-deficient mice.

We observed that spontaneous erythematous lesions occurred on the skin and that some animals developed spontaneous localized diffuse alopecia. Histologically cutaneous lesions showed marked acanthosis, dermal edema and localized vacuolar degeneration of the basal cell layer as well as a dermal infiltrate consisting mainly of myeloid CD68+ mononuclear cells. Immunofluorescence analysis showed a stronger deposition of immune complexes and complement c 3 along the dermo-epidermal junction in mutant versus wild type animals also in uninvolved murine skin. Moreover, deposition of the interferon-induced chemokine CXCL10 was prominent in lesional skin of TREX1-deficient mice and similar to expression of CXCL10 in cutaneous lesions of lupus patients with mutations in TREX1.

These data demonstrate that TREX1-deficient mice develop spontaneous cutaneous inflammation reminiscent of human lupus lesions and support the role of TREX1 mutations in the pathogenesis of cutaneous lupus.

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T cells with an autoreactive phenotype and a deregulated production of reactive oxygen species occur as a feature of immunosenescence in old mice

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Aging of the immune system, termed 'immunosenescence', is characterized by a functional decline leading to an onward immunodeficiency ('hyporeactive'). Notably, regulatory mechanisms also decline with aging resulting in a paradoxical 'hyperreactive' immune response. Concurrently, tumor, infection, and autoimmune disease occur with high prevalence in the elderly. Interestingly, a senescent T cell repertoire, phenotype and function alters immunity not only in the elderly but also in patients suffering from systemic chronic inflammation.

We here precisely characterize distinct age-dependent T cell subpopulations showing a profile of dysfunctional and/or autoreactive T cells in peripheral lymphoid organs of young, adult and old mice (3–6, 9–12 and 18–24 months-old C57BL/6, $n \geq 5$). The majority of the CD4 T cell subset showed an age-related reduction of CD27 and the CD28 costimulatory receptor and down-regulation of CD5. Only a minority of the CD4 T cell subset showed a CD27(low) CD28(hi) CD5(hi) phenotype indicating homeostatic expansion, which typically occurs due to a reduced thymic T cell output with age. Also CD8 T cells and CD4- CD8- DN T cells had this age-related CD27(low) CD28(hi) CD5(hi) phenotype suggesting these cells have an age-related increase in self reactivity. Interestingly, the observed unconventional CD4-CD8- DN T cells phenotypically resembled a peripheral T cell subset

previously shown to accumulate rapidly in lymph nodes of a mouse model of leukocyte-adhesion deficiency syndrome 1 (LAD1), a primary immunodeficiency syndrome with a premature immune aging phenotype.
Recent reports indicate that imbalanced levels of reactive oxygen species (ROS) contribute critically to driving chronic inflammation and immunosenescence in an ambiguous fashion, with a yet not fully understood role in T cells. Therefore, we used high-throughput six-channel fluorescence FACS to further analyze the age-depedent CD27(low) CD28(hi) CD5(hi) T cells regarding their ability of ROS induction in an next step. As a result, CD27(low) CD28(hi) CD5(hi) T cells showed a markedly increased ROS response to the mitochondrial electron chain complex inhibitor rotenone *ex vivo* measuring increased O2- radicals (by means of the oxidation of dehydroethidium) and H2O2 (by oxidation of dichlorofluoresceine diacetate) as compared to phenotypically normal nave and antigen-experienced control T cells. The functional role of the highly active ROS production in this T cell subset will now be tested in antigen-specific activation assays *in vitro* as well as using highly specific ROS luminescence imaging *in vivo* to unravel ROS biology in T cells of mice at different age groups.
In summary, our data reveal a substantial increase of distinct T cell subsets characterized by a highly detailed phenotypic profile in old mice showing strong characteristics of dysfunction and autoreactivity. This is further supported by a T cell phenotype-dependent altered ROS profile. Our data may contribute to clarify important aspects of an age-associated dysfunction of T cells in an aging immune system and will also give insight into pathomechanisms that unite or separate immunosenescence from exhaustion by chronic inflammation and/or immunodeficiency.

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Human type VII collagen harbors multiple pathogenically relevant epitopes

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Epidermolysis bullosa acquisita (EBA) is an autoimmune blistering disease of skin and mucous membranes, characterized by autoantibodies against type VII collagen (COL7), a major component of anchoring fibrils of the skin. Several clinical phenotypes of EBA have been described, including mechano-bullous and inflammatory variants. Most EBA patients' sera react with the non-collagenous (NC)-1 domain of COL7. However, it remained unclear, if binding to only certain epitopes induces EBA, and if binding to specific epitopes within the NC-1 domain causes the different phenotypes of EBA. Therefore, we generated 10 recombinant overlapping proteins covering the entire NC1 domain. Sera from 69 EBA patients were analyzed for IgG reactivity with these recombinant proteins by immunoblotting. Most sera recognized clusters of epitopes throughout the NC1 domain. No significant correlation was detected between antibody specificity and clinical phenotype. However, a strong dependency of epitope distribution on gender and age was noticed. To study pathogenicity of antibodies specific to the different subdomains of NC1, rabbit polyclonal antibodies to different epitopes were generated. Interestingly, all antibodies, regardless of their specificity, caused dermal-epidermal separation *ex vivo* when incubated with cryosections of human skin and subsequently with leukocytes of healthy volunteers. In the final set of experiments, antibodies against two subdomains of NC1 domain were affinity-purified and injected into COL7m−/−,h+ mice which carry the homozygous null mutations of mouse Col7a1 genes and the transgene of human COL7A1 with the ubiquitous CMV promoter. Both antibodies caused specific clinical and histological changes in mice. These observations add to our understanding of the autoimmune response in EBA and document pathogenic effects of autoantibodies directed to several epitopes within the NC1 domain, both *ex vivo* and *in vivo*. In addition the results suggest that the different clinical phenotypes of EBA do not depend on antibody specificity, but may rather depend on the ability to mount an inflammatory response subsequent to IgG binding to the dermal-epidermal junction.

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Effects of AhR activation on the maturation of human Langerhans cells

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The aryl hydrocarbon receptor (AhR) is a ligand activated transcription factor that has been implicated in immunology. It has been shown that AhR activation can suppress immunity in a mouse model of allergic asthma as well as pancreatic island transplantation and can suppress or induce immunity in a mouse model of EAE. T-cells and dendritic cells were affected by addition of AhR ligands.
We showed before that the AhR ligand VAF347 can interfere with Langerhans cell differentiation in a model of LC differentiation from CD34+ hematopoetic stem cells.
We were now interested in the effect of the endogenous AhR ligand FICZ on Langerhans cells. FICZ has been shown to be present in the skin upon UV-irradiation. Therefore, due to their localisation in the epidermis, Langerhans cells are prone to get in contact with FICZ. Further we were interested in the function of AhR ligand treated LCs.
We found that FICZ, like VAF347, inhibited differentiation of LCs differentiated from human CD34+ hematopoetic stem cells. LCs that were differentiated in the presence of AhR ligands showed a decreased ability to mature, i.e. a decreased upregulation of CD80, CD83, and CD86 upon stimulation with PGN. Interestingly, expression of HLA-DR and CD40 was slightly increased. However, AhR-ligand treated LCs did not show a regulatory phenotype as shown for AhR-ligand treated dendritic cells. There was no induction of IL- 10 production and of the regulatory surface molecules PDL-1 or PDL-2.
Therefore, Langerhans cells react differently towards AhR stimulation compared to dendritic cells. This is interesting as Langerhans cells are prone to get in contact with AhR ligands due to their exposed localisation in the epidermis.

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Th17-cell produced GM-CSF: a novel crucial element of the pathogenetic cascade in psoriasis?

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An essential pathogenetic role of granulocyte macrophage colony-stimulating factor (GM-CSF) secreted by Th17 cells has recently been demonstrated for EAE, a murine model of multiple sclerosis. However, the sources and significance of this mediator in common chronic inflammatory diseases in humans, such as psoriasis, are still completely unknown. In the first part of our current study we investigated the GM-CSF-production by *in vitro* generated human Th1-, Th2-, Th17-, Th22-, Treg-, and Tr1-cells. Surprisingly, we found a strong GM-CSF mRNA expression and protein production by Th22- and in particular by Th1-cells. In contrast, Th17-cells showed the lowest GM-CSF mRNA and protein levels among all analyzed Th-cell subpopulations. In line, about 50% of GM-CSF-positive blood effector/memory Th-cells were simultaneously positive for IFN-γ after short *ex vivo* stimulation, but <10% of them co-produced IL-17A. Importantly, also *in vivo* developed effector/memory Th1-cells isolated by means of flow cytometry based on differential chemokine receptor surface expression produced more GM-CSF than respective isolated Th17-cells after long *ex vivo* stimulation. In the next part of our work we investigated the mechanism underlying the limited GM-CSF production by Th17-cells and identified a strong dose-dependent inhibitory effect of TFG-β that was independent of the used generation protocol. Interestingly, the presence of the anti-inflammatory cytokine IL-10 during the polarization period did not have any influence on Th17-cell GM-CSF-production. Next, we investigated the importance of different signaling events downstream of TCR and CD28 for the GM-CSF production in established Th1-cells. Furthermore, we learned that neither the Th1- nor the Th2-cell prototypical cytokines IFN-γ and IL-4 influenced GM-CSF-production by established Th1-cells. Finally, we detected elevated GM-CSF level in diseased skin of patients suffering from psoriasis compared to skin of healthy donors. In line with the known GM-CSF-effects on cytokine production by myeloid cells, the level of GM-CSF in psoriatic lesions positively correlated with the level of the Th17- and Th22-pathway-promoting cytokines IL-23 and TNF-α. Our data suggest that, independent of its sources which may differ to the murine system, GM-CSF may play an important role in psoriasis pathogenesis.

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T-cell receptor repertoire in acute skin infection

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In the majority of human T cells of peripheral blood and lymphoid organs, the T-cell receptor (TCR) recognition unit consists of $\alpha\beta$ heterodimers ($\gamma\delta$ heterodimers are usually below 10% of the T cells). The transducing unit is part of the CD3 complex. Monoclonal antibodies against the 24 most common human beta chain variants which represent about 2/3 of the repertoire of healthy donors were used in a TCR Vβ repertoire analysis kit to study the TCR repertoire of CD4+ and CD8+T cells of 26 patients with cellulitis admitted to our hospital. Investigating CD4+ T cells, in 10 patient samples (38%) a normal distribution of the 24 β-chain variants was observed. In comparison to healthy donors, eight patients (31%) showed one of the 24 beta chains above normal range. Seven patients (27%) showed oligoclonal (reactive) distribution of the 24 beta chains without any specific pattern.
A different picture was found in CD8+ T cells: 21 patients (81%) showed oligoclonal (reactive) distribution, among them four patients with expansion of four different β-chain variants. Vβ16 chain was detected above range in 18 of all 26 cases (69%), other expanded beta chains were distributed in a heterogeneous pattern. In three patients a predominance (>68%) of one single β chain was found (Vβ3, Vβ5.1 and Vβ7.2). Only in two patients a normal distribution of the 24 β-chain variants was observed. Three patients showed one of the 24 beta chains above normal range.
Taken together, to our surprise, in more than two thirds of patients no response in the CD4+ T-cell receptor repertoire was detected during an acute skin infection like cellulitis. In 27% of cases, CD4+ T-cell receptor repertoire showed oligoclonal expansion with no specific pattern. In contrast, the CD8+ T-cell receptor repertoire showed a reactive oligoclonal distribution in 81% with expansion of Vβ16 chain in the majority of samples.

P201

Activity of ER aminopeptidase 1 in malignant melanoma influences the strength of CD8+ T cell responses against autologous tumor cells

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The knowledge of HLA class I-restricted tumor antigen epitopes recognized by cytotoxic CD8+ T lymphocytes (CTL) is currently exploited in immunotherapy of malignant melanoma, in vaccination as well as adoptive T cell therapy. However, the generation of CTL epitopes in tumor cells has been studied only marginally. In general, the multi-catalytic proteasome complex cleaves a tumor antigen into peptide fragments of different length, thereby defining the C-terminus for the majority of peptides presented by HLA class I molecules. While some of the proteasome products are of the correct size for direct HLA class I binding others are *N*-terminally elongated peptide precursors that require further trimming by aminopeptidases. Recent data suggested that the ER aminopeptidase 1 (ERAP1) might be of specific relevance for the efficient generation of HLA class binding peptides in tumors as it was demonstrated that low *in situ* expression of ERAP1 is associated with reduced overall survival in cervical carcinoma. So far, the significance of ERAP1 activity for the overall recognition of tumor cells by CD8+ T cells has not been determined. To do so, we employed the autologous tumor-CD8+ T cell system of patient Ma-Mel-86. Melanoma cell lines (Ma-Mel-86a, Ma-Mel-86c) were established from different metastasis of this patient, in which expression of ERAP1 was downregulated by shRNA. ERAP1 knockdown, confirmed by quantitative RT-PCR and Western Blot analysis, did not influence the expression level of total HLA class I molecules in comparison to control cells (mock transfectants). To determine the impact of ERAP1 knockdown on T cell stimulation Ma-Mel-86a cells were used to repeatedly stimulate autologous CD8+ T cells. Subsequently, the T cells were analyzed for their capability to recognize Ma-Mel-86a (mock transfectants) in comparison to ERAP1-shRNA transfected Ma-Mel-86a cells. Interestingly, ERAP1 knockdown impaired the recognition of melanoma cells by autologous bulk CTL, indicating that ERAP1 indeed impacts the epitope presentation by this tumor cells. To get knowledge about ERAP1-dependent tumor antigen epitopes, autologous T cell clones of known specificity were incubated with the indicated melanoma target cells. Three of four analyzed T cell clones were significantly less activated by ERAP1-knockdown melanoma cells. In conclusion, ERAP1 activity in melanoma cells influences T cell activation via the amount of the generated specific epitopes suggesting that tumor cells might escape from efficient T cell recognition by altering ERAP1 expression.

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T cell activation in draining lymph nodes during experimental epidermolysis bullosa acquisita does not affect skin inflammation

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Epidermolysis bullosa acquisita (EBA) is an autoimmune bullous dermatosis (AIBD) which is characterized by autoantibodies against type VII collagen (COL7), a structural protein of the skin. In this study, antibodies against murine COL7 were generated in rabbits and transferred into mice. As described previously, binding of these autoantibodies to murine skin induced skin blistering duplicating the findings in EBA patients. Interestingly, concurrently with blister formation in the skin, the size of draining lymph nodes increased dramatically. Immunohistochemical investigations revealed an increased T cell proliferation in the T cell zone of draining lymph nodes. By analyzing the gene expression pattern, we found that the expression of inflammatory genes like Fcγ receptor IV was significantly increased in draining lymph nodes. In addition, the expression of the Th1 cytokine IFN-γ also increased whereas expression of IL-4 decreased. No change in the gene expression pattern was

found after injection of nonpathogenic rabbit IgG suggesting that only the injection of antibodies directed against skin-derived type VII collagen stimulates a Th1 differentiation in draining lymph nodes. Since T cells are found in skin lesions, we asked whether lymph node-derived Th1 cells would play a role in the inflammatory events observed in the skin of EBA mice. To block T cell migration out of the lymph node, we used the sphingosine-1-phosphate receptor antagonist FTY720 (Fingolimod). Treatment with FTY720 led to a strong decrease of T cell numbers in the peripheral blood but had no effect on both the intensity of skin blistering and the expression of inflammation associated cytokine mRNAs in skin lesions. We conclude that the inflammatory events in the skin have an impact on T cell polarization in the draining lymph node. However, the Th1 polarization within the lymph node has no effect on the intensity of the skin disease. Our data indicate that the communication between skin and lymph node in experimental EBA proceeds mainly from skin to lymph node and not from lymph node to skin.

P203 (O26)

cAMP regulated proinflammatory programming of human 6-sulpho LacNAC (slan) dendritic cells

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SlanDCs are discrete population of dermal and blood dendritic cells (DCs) with relevance in driving inflammatory immune responses in psoriasis, lupus and HIV. We here asked for the molecular mechanisms responsible for the pro-inflammatory function of this cell type. We observed a transient increase in the intracellular concentration of cAMP during early stage of spontaneous slanDC maturation. We were keen to understand its relevance for upregulating maturation markers, and its modulation of the cytokine production and T cell programming. Inhibition of the early cAMP peak by blocking the cAMP producing enzyme Adenylate cyclase by DDA inhibited upregulation of markers of mature DCs (CD83, CD86 and HLA-DR), and consequently, DC maturation was restored by adding exogenous dibutyryl cAMP (dbcAMP). Stabilization of short-lived cAMP by PGE2, dbc-cAMP, forskolin or by inhibition of cAMP degradation by blocking PDE4 did not influence phenotypic maturation but completely inhibited the LPS/IFN? induced IL-12 and TNF? production and concurrent elevation of IL-10 production. Stabilization of cAMP moreover reduced Th1 programming capacity of slanDC. The cAMP stabilization led to accumulation of HO-1 and phospho p38MAPK intracellular proteins which got degraded during the course of maturation. We could identify the relevance Cox-2 and MEK-1 proteins in cAMP-mediated inhibition of IL-12 and TNF-production in slanDCs. These results demonstrate that transient upregulation of cAMP drives the phenotypic maturation of slanDCs and that a sustained cAMP signal restrains their high pro-inflammatory capacity which is mediated through upregulation of both Cox2 and MEK-1 proteins. The dual role of cAMP for slanDC maturation revealed in this study may explain seemingly contradictory findings of the inhibitory and stimulatory function of cAMP on DCs maturation reported in the past. Furthermore, the results shed light on the potential mode of action of therapeutic PDE4 inhibitors that are currently under study for the treatment of psoriasis and atopic dermatitis.

P204 (O16)

Neutrophils play a central role in the sensitization and elicitation phase of contact hypersensitivity

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Allergic contact dermatitis (ACD) is a T cell mediated inflammatory skin disease. The innate immune system plays a crucial role in orchestrating the inflammatory response but the mechanisms leading to the induction of innate inflammation in this disease are still not understood in depth.

We investigated the role of neutrophils in the contact hypersensitivity (CHS) model, the mouse model of ACD. Upon contact allergen treatment we observed a rapid and profound neutrophil infiltration of the skin. We used a model of specific neutrophil depletion and a strain of genetically neutrophil deficient mice. We found that both- the sensitization of nave mice and the elicitation of the CHS response in sensitized mice are dependent on neutrophils. During the sensitization phase we observed *in vivo* numerous contact allergen induced signs of inflammation such as ROS production, gelatinase activity and the production of various chemokines and cytokines. In neutrophil depleted or deficient mice, the levels of all of these inflammatory markers were significantly decreased. We observed that in the absence of neutrophils the allergen induced migration of dendritic cells from the skin to the draining lymph nodes was absent. Restimulation of T cells from mice *ex vivo* showed, that if neutrophils were absent at the time point of sensitization, the T cells would respond to restimulation with the contact allergen by drastically reduced production of interferon-γ compared to the controls. This indicates that T cell priming depends on the contribution of neutrophils. Among the cytokines which were depending the strongest on neutrophils was Interleukin(IL)1β. To test the physiological relevance of the IL-1β in this setting, we used recombinant IL-1β which we administered intradermally prior to sensitization. Administration of recombinant IL-1β could restore the sensitization process in neutrophil depleted mice.

Passive CHS experiments revealed, that in the elicitation phase, neutrophils are needed to recruit effector T cells to the challenged ear skin. Without the contribution of neutrophils the CHS response can not be elicited and the typical symptoms do not occur.

In summary, we here demonstrate neutrophils to be key mediators of innate inflammation in CHS. Without their contribution, dendritic cell emigration from the skin is impaired and T cell priming is deficient. During the elicitation phase, neutrophils are needed to recruit effector T cells to the skin. Modulating neutrophil activation and recruitment might be a future therapeutic target for the treatment of ACD.

Infectious Diseases

P205 (O31)

HPV 16 activates the AIM2 inflammasome in keratinocytes

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HPV are small, non-enveloped DNA viruses that selectively infect keratinocytes in stratified epithelia. To date, over 150 genotypes have been identified. HPV subtypes are further characterized as 'low-risk' and 'high-risk' viruses according to their oncogenic potential. E.g. the virus types 6 and 11 lead to the development of benign cutaneous warts whereas the virus types 16 and 18 can cause epithelial carcinoma after long-standing infection.

After an initial infection many patients clear HPV, however in some patients such as HIV positive individuals HPV infection persists and HPV can act as a carcinogen. The mechanisms behind the effective clearing of HPV infections are yet unknown. Dysfunctional innate immune responses to HPV infection could be involved in the ineffective clearing of the virus. Indeed, HPV infection deregulates the cellular network comprising chemotactic and pro-inflammatory genes, and downregulates the transcription of some pro-inflammatory genes in keratinocytes.

HPV are double-stranded DNA (dsDNA) viruses and recently a sensor for cytosolic dsDNA named AIM2 was described. Binding of viral DNA leads to AIM2 inflammasome activation and subsequent IL-1β release. IL-1β plays a key role in the further activation of the local innate immune response. Another important dsDNA sensor is IFI16. This molecule is primarly localized in the nucleus, but can translocalize to the cytosol on danger signals. dsDNA which binds to IFI16 leads to release of IFN-β which also plays an important role in the primary viral response.

In this study, we investigated, how early infection of HPV16 modulates the innate immune response of keratinocytes. We observed that in chronic inflammatory HPV lesions the cytosolic DNA sensor AIM2 is highly expressed. In addition, active IL-1β and cleaved caspase-1 were detected in HPV infected skin. In further *in vitro* experiments, HPV triggered IL-1β and IL-18 release via the AIM2 inflammasome in normal human epidermal keratinocytes (NHEK). Additionally, when AIM2 was blocked HPV induced high amounts of IFN-β through IFI16. In contrast, blocking of IFI16 leads to higher release of IL-1β, but not IL-18, suggesting that IFI16 and AIM2 interact in the immune response to DNA viruses.

In sum, we could identify novel aspects in HPV 16 induced immune responses. The activation of local innate immunity may play an important role for the efficient clearing of the virus. Eventually, understanding the mechanisms of HPV induced inflammasome activation in HPV-infected skin could lead to innovative strategies for the prevention and treatment of HPV infections.

P206 (O32)

Mast cells control skin wound infections with *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa (PA), a ubiquitous environmental Gram-negative bacterium, is one of the leading causes of severe nosocomial skin wound infections. Due to increasing resistance to common antibiotics, this pathogen poses a major threat, especially to immunocompromised patients therefore new therapeutic approaches are needed. Since mast cells (MC) are situated at the borders to the external environment like the skin their localisation allows them to be among the first inflammatory cells to encounter and act against invading pathogens. The aim of the present study was to examine the effects of MCs on bacterial skin wound infections with PA. We found that MCs are beneficial for wound healing and clearance of PA in a model of full thickness skin wound infections. Using MC-deficient mice (KitW/KitW-v) we detected reduced wound closure (26.2% 8.6 $P < 0.05$) and 15-fold increase of PA numbers in wound tissues as compared to wild type mice. Reconstitution of KitW/KitW-v mice with bone marrow-derived MCs before wounding resulted in normalisation of wound healing. To analyse the molecular mechanisms responsible for these antibacterial and wound healing promoting effects of MCs, we looked at MC-PA interaction *in vitro* and found only a minor direct antibacterial capacity of MCs but a crucial interaction between MCs and cells of the epidermal skin compartment resulting in the reduction of bacterial burden. These findings demonstrate an important role of MCs in controlling skin wound infections by PA. This supports the function of MCs as key players in innate immune responses to bacteria in a clinically relevant infection model.

P207 (O22)

Dermal dendritic cells promote healing and induce protective immunity against Leishmania major

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Defence mechanisms against pathogens are exerted by different skin-derived dendritic cell (DC) subsets, including dermal DC (dDC) and Langerhans cells (LC). In experimental cutaneous leishmaniasis, LC are negative regulators of the anti-Leishmania (L.) response, and - as a consequence of this - other skin migratory DC subtypes mediate the induction of protective immunity. Chemokine receptor CCR7 is highly expressed on mature DC as well as on naive and central memory T cells, and CCR7 is crucial for DC migration from peripheral tissues to the draining lymph nodes (LN). In the present study, we used CCR7-deficient which mice have a severe defect in skin DC migration to the draining LN. We analyzed CCR7-deficient and C57BL/6 control mice in the L. major low dose infection model, including inoculation of only 1000 infectious-stage promastigotes into the dermis. Compared to controls, lesion development was significantly enhanced in CCR7-deficient mice. In line, parasite loads were larger in L. major-infected ears of knock-out mice. In week 6 post infection, we analyzed the antigen-specific cytokine profile of LN cells restimulated with soluble Leishmania antigen (SLA). Here, production of the T helper (Th) 1 cytokine IFN-gamma was significantly reduced in CCR7-deficient mice as compared to wild types. Additionally, we compared immigration of inflammatory cells in infected ears and LN of both mouse strains. The frequency of CD4+ Foxp3+ regulatory T cells was increased in LN of CCR7-deficient mice. On the other hand, the number of CD8+ cytotoxic T (Tc)1 cells was decreased. In addition, influx of CD11b+ Ly6Cint Ly6G+ neutrophils was enhanced in LN as well as in infected ears of CCR7-deficient mice, and total DC frequencies in the infected skin were higher in knock out mice. In an independent experiment, we utilized CD11c-DTR (donor) - C57BL/6 (recipient) bone marrow (BM) chimeras to deplete CD11c+ DC after administration of diphtheria toxin (DT), but not the radio-resistant LC. Here, CD11c-DTR BM was used to reconstitute lethally irradiated C57BL/6 host mice. Radio-resistant epidermal LC remain of host (C57BL/6) origin, whereas all other DC subtypes (including dDC) are radiosensitive and subsequently replaced by donor-derived cells. During low dose infection with L. major, these DT-treated BM chimeras developed exacerbated disease with larger lesion volumes as compared to PBS-treated controls, indicating that CD11c+ dDC are required to promote the induction of efficient protective immunity. In summary, both experimental approaches, the depletion or the prevention of migration of dermal DC, led to worsening of the disease, indicating that these skin DC are important for T cell priming in the LN to finally resolve L. major infections.

P208

Identification of CD4+ and/or CD8+ T cell activating peptides from a LACK (Leishmania of homologue for receptor of activated c kinase) library

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Healing of Leishmania (L.) major infections is based on Th1/Tc1 immunity and requires secretion of IFNγ from both CD8+ and CD4+ T cells. No vaccine exists against this human pathogen. Interestingly, so far only one single peptide epitope from LACK (Leishmania of homologue for receptor of activated c kinase) protein is known, which activates CD4+ T cells. LACK is expressed in both life-forms of L. major - infectious-stage promastigotes and intracellular amastigotes. Together with an adjuvant, LACK cDNA and/or protein were shown to effectively vaccinate against progressive leishmaniasis. Now, to analyse CD4-peptide reactivity *in vitro*, dendritic cells (DC) were isolated and cultured from nave BALB/c mice. On day 6, primed CD4+ T cells from infected mice and peptide-loaded DC were cocultured for 48 h. After 2 days, supernatants were analysed for the release of IFNγ, IL-4 and IL-10. As expected, the LACK-peptide induced CD4+ T cells to secrete high amounts of IL-4 and IL-10 compared to control. Release of IFNγ was not observed. To identify potential peptides derived from LACK protein capable of inducing higher secretion of IFNγ and associated with activity during vaccination, we created an overlapping peptide library of the 36 kDa LACK protein consisting of 50 peptides: 47 peptides as overlapping 15 mers, and three peptides as control peptides - SIINFEKL, GYKDGNEYI (both CD8+ epitopes) and the known CD4 peptide. To analyse peptide-reactivity *in*

vitro, DC were isolated from nave C57BL/6 mice. After six days, peptide-pulsed DC were cocultured with primed CD4+ and CD8+ from infected mice for 48 h. After 2 days, supernatants were assayed for IFNγ, IL-4 and IL-10 secretion. Interestingly, two peptides induced stronger IFNγ release from CD8+ T cells compared to controls. IL-4 and IL-10 secretion was enhanced when CD8+ T cells were stimulated with other peptides. CD4+ T cells did not secrete IFNγ when activated with any peptide, whereas several peptides including the known CD4 LACK peptide induced secretion of IL-4 and IL-10. Next, those two peptides inducing IFNγ secretion from CD8+ T cells *in vitro* will now be analysed for their immunizing potential *in vivo* in C57BL/6 and BALB/c mice. In summary, an identification of CD4+ and CD8+ epitopes from the LACK protein which activate CD8+ and/or CD4+ T cells *in vivo* would aid the development of a vaccine against this important human pathogen.

P209

Soluble Leishmania antigen as potential vaccine against Leishmania major *in vivo*

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The human pathogenic parasite Leishmania (L.) major holds responsible for the development of human cutaneous leishmaniasis (CL). Healing of this infectious disease is mediated by the release of interferon (IFN)γ by both antigen-specific CD4+ T helper (Th) 1 and CD8+ cytotoxic T (Tc) 1 cells, but an effective vaccine against CL does not exist. However, its development should be feasible, since immunity of immunocompetent hosts develops after resolution of infection. Due to increased morbidity of affected individuals with e.g. co-infections, such a vaccine would be of high interest. Therefore, we investigated the immunogenic effect of Leishmania lysate (SLA) *in vivo* with the aim to identify and characterize L. major-specific immunogenic antigens. First, we immunized C57BL/6 mice s.c. in one ear with different doses of SLA prepared of L. major promastigotes ranging from 10E3 up to 10E6 parasites in either the presence or absence of an adjuvant. One week later, infections were initiated with live parasites in the alternate ear, and lesion size was measured weekly. Surprisingly, we observed a strong dose-dependent difference in their vaccination potential, as only mice treated with low SLA doses without adjuvant showed dramatically smaller lesions and healed within a shorter time period in contrast to groups treated with high doses of SLA. Interestingly, this effect was reversed in mice after injection of SLA + CpG. Here, efficient vaccination was achieved using CpG, but only in those mice injected with high doses of SLA, whereas CpG in low SLA vaccinations did not exhibit a beneficial effect compared to equal SLA doses alone. Second, a similar dose- and adjuvant-dependent behaviour of lesion progression post infection was also observed after a prime/boost/boost (P/B/B) approach with different amounts of isolated soluble proteins (SP) from SLA compared to whole parasite lysates. SP were revealed after differential centrifugation from SLA. Here, immunization with low amounts of SP + CpG caused smaller lesion sizes compared to those of mice treated with high SP amounts + CpG. Finally, complete Freund's adjuvant was more potent as adjuvant than CpG. The complexity of injected Leishmania-specific proteins may form the basis for these antigenic dose-dependent vaccination effects, as some antigens might exert a stronger binding affinity to specific TCRs in a dose-dependent manner. Therefore, protective T cell reactivity *in vivo* may strongly depend on the dominance and binding affinity of L. major-specific antigens during the process of antigen processing and presentation. Since immunization studies in mice showed promising results with selected recombinant Leishmania-specific antigens in combination with an adjuvant, the discovery of additional protective proteins *in vitro* and *in vivo* may aid a better understanding of T cell-mediated protection against CL.

P210

Dendrititc cells, macrophages and neutrophils in murine cutaneous leishmaniasis: subtypes and immigration kinetics during physiological low-dose infection

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Leishmaniasis is an infectious disease caused by a protozoan parasite transmitted by the bite of a sand fly. As a first innate immune response, skin resident macrophages (MΦ) ingest promastigote parasites which transform to obligate intracellular amastigotes and replicate inside of these cells. Also, neutrophils (PMN) exercise their phagocytic function. Parasites are released by rupture or exocytosis and infect migratory inflammatory cells. Dendritic cells (DC) incorporate amastigotes and initiate T-cell responses via IL-12. This leads to production of IFNγ and resistance of C57BL/6 mice via a Th1 response, whereas susceptible BALB/c mice show predominant Th2 development with IL-4 and IL-10. Now, we intended to assess details in the immigration kinetics of PMN, MΦ and DC in these two mice strains after L. major infection. BALB/c and C57BL/6 were infected intradermally with 1000 metacyclic promastigotes into ears and lesion development was measured weekly. In weeks 3, 6, 9 and 12 post infection, we assessed infected skin and draining lymph nodes by FACS analysis and a 7-colour staining protocol. First, we used markers against CD11b/ Ly6C/ Ly6G/ Gr-1/ CD11c/ MHC class II and F4/80. We preselected for myeloid cells by CD11b+ gating, and studied three distinct populations as follows: A) PMN were classified as Ly6G+, Ly6Clow, Gr-1+ and 7/4+; B) inflammatory monocytes (Mo) as Ly6Chigh and Ly6G-; C) a population of Ly6C- and Ly6G- cells, containing DC (MHC class II+/ CD11c+) and MΦ (F4/80+). First, the number of myeloid cells (CD11b+) increased in infected skin from week 0 to 6 and decreased in week 9 in C57BL/6 mice, in contrast to BALB/c mice, where it continued to increase. PMN (population A) in ear samples showed an interesting progression particular in BALB/c mice. We detected the highest numbers in week 9, whereas in resistant mice, PMN numbers slightly increased after infection and stayed a little above baseline level over the entire infection period. Interestingly, in resistant mice, we found a continuous increase of population B until week 12, but in BALB/c mice, numbers of inflammatory Mo remained significantly lower. In control mice, 70 to 80% of all CD11b+ cells belonged to this double negative population. Post infection, their number gradually decreased parallel to the increase of immigrated cells (populations A and B) into infected skin. In summary, in earlier studies, PMN were identified to play a role for the susceptibility of BALB/c mice, and an increase of inflammatory Mo was suggested to be important for resistance in leishmaniasis. In line, our noticeable differences in the kinetics of PMN and inflammatory Mo between the two mouse strains suggest to strongly focus on these cells in future therapeutic studies.

P211

TNF-α regulates the vitamin D-dependent antimicrobial response in human monocytes

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Reactivation of tuberculosis is a major adverse event when treating with TNF-α inhibitors in dermatology, however the role of TNF-α in M. tuberculosis infection in humans remains unclear. In this study, we investigate the role of TNF-α on the vitamin D antimicrobial pathway, which involves the induction of the antimicrobial peptides cathelicidin and DEFB4, as well as the cathelicidin-dependent induction of autophagy. Using primary human monocytes cultured in vitamin D sufficient conditions, we found that TNF-α induced cathelicidin and DEFB4 antimicrobial peptide expression. However, TNF-α failed to induce autophagy in human monocytes, suggesting the induction of cathelicidin and autophagy is not always linked. Furthermore, TNF-α inhibited the IFN-γ-mediated induction of cathelicidin and DEFB4 gene expression, as well as autophagy. Our data suggest that TNF-α by itself partially activates the vitamin D-dependent antimicrobial pathway, but can also inhibit the IFN-γ-mediated protective host response.

P212

Chronic unilateral migratory periorbital nodules and redness

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A 39-year-old Caucasian female patient was admitted to our ward with skin nodules in left periorbital area, which were migratory and persistent for 1 year. The patient had no fever, night sweat or weight loss. CBC including differential (no leukocytosis or eosinophilia) and chemistry (no increase in C - reactive protein) were in normal range. Travel anamnesis revealed frequent trips to India (with total stay of 1.5 year and last trips being 18 and 12 months prior to hospital admission) but not to Africa. The family history was inconspicuous and she was otherwise healthy. A day before admission the periorbital area was erythematous and she observed a moving subconjunctival object. After ophthalmological assessment the decision for conjunctical incision and physical removal of the object was made. During this process a 13.5 cm long worm was isolated. Phylogenetical analysis of the worm showed a female Dirofilaria repens. A topical anti-inflammatory therapy with Ultracortenol® (prednisolone) eye drops was initiated. Blood analysis (for the presence of worm and worm eggs) as well as detailed clinical/radiological evaluation of the patient confirmed that there were no systemic manifestations of the disease.

Dirofilaria (Nochtiella) repens (Nematoda: Onchocercidae) is a subcutaneous parasite of dogs and other carnivorous animals, with human acting as incidental hosts. Dirofilaria repens occurs endemically in warm climates on various continents.

The frequency of reports is increasing in literature during the last few years and is considered by some authors as an emerging zoonosis.

P213

Post-septic immune-suppression following gram positive sepsis is mediated by TLR dependent induction of myeloid derived suppressor cells

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Systemic bacterial infection and consecutive sepsis is a leading cause of death in Germany and Gram+ bacteria are a major cause. Treatment of acute sepsis has been improved and many patients survive primary infection. However, severe secondary infections due to post-septic immune suppression are still associated with high mortality in part because underlying mechanisms are poorly understood.

To this end, we established a mouse model of Gram+ sepsis. C57BL/6 or BALB/c mice were intravenously infected with high doses of *Staphylococcus aureus* SA113. Animal weight as indicator of sepsis severity and bacterial CFU in the kidneys were determined as well as several immune parameters at different time points. Importantly, the post-septic immune status was assessed by determining a cutaneous T-cell mediated recall response to FITC (FITC-contact hypersensitivity; CHS). Indeed, post-septic immune suppression was established in these mice, because FITC-CHS was significantly reduced in comparison to the uninfected control. Among several immune parameters analyzed, the most striking finding was a massive expansion of the Gr-1+ CD11b+ so called myeloid derived suppressor cells (MDSC) in septic mice. To assess whether MDSC induction by SA113 sepsis contributes to post-septic immune-suppression, MDSC were first analyzed *ex vivo*: MDSC completely blocked T-cell activation with suppressive functions of MDSC dependent on reactive oxygen species (ROS). Next MDSC were eliminated *in vivo* using a depleting anti-Gr-1 antibody and, strikingly, MDSC depletion completely abrogated post-septic immune suppression of FITC-CHS. Analyzing underlying mechanisms, we found that MDSC induction *in vivo* was independent of either IFN-γ, TNF, IL-4, or IL-10. In contrast, MDSC induction was completely abrogated when mice deficient in common TLR-adaptor protein MyD88 were infected. Further analyses demonstrated that MDSC induction by Gram+ bacteria depended on both, TLR2- and TLR9-signaling, whereas NOD-2 was not involved.

In summary, we show for the first time post-septic immune suppression after Gram+ sepsis to be mediated by MDSC induced via MyD88 and TLR2/9 signaling.

P214

A modulatory role for the aryl hydrocarbon receptor in experimental leishmaniasis

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In experimental L. major infection a Th1 response in C57BL/6 mice results in resistance while a Th2 response is associated with susceptibility in BALB/c mice. The development of Th1/Th2 immune responses is driven by the early cytokine milieu in the infected tissue. We have previously shown that both keratinocytes and phagocytes contribute considerably to the immunological events preceding those in lymph nodes. Skin macrophages are among the first cells to come into contact with L. major. To analyse their contribution to the early cytokine milieu we studied L. major induced gene expression in macrophages 24h after *in vitro* Infection. Differences in the skin cytokine milieu between resistant and susceptible mice strains are responsible for several aspects of resistance to L. major, most notably for signals from the innate part of the immune response that determine Th- cell differentiation. We found that the transcription factor aryl hydrocarbon receptor (AhR) is considerably stronger upregulated in C57BL/6 macrophages than in BALB/c macrophages infected with L. major. AhR is involved in several processes in the skin, for example reaction to UV radiation, retinoic acid signalling and reducing dioxin toxicity. It is also important in several types of both adaptive and innate immune cells, for example differentiation of Th17 cells or negative regulation of macrophage response to LPS.

Thus strain specific differences in AhR expression in skin resident macrophages have the potential to influence development of resistance to infection, e.g. by modifying the early cytokine milieu in L. major infected skin. We performed *in vitro* experiments with knock out macrophages and AhR ligands in order to investigate the contribution of AhR to macrophage cytokine secretion caused by Leishmania infection. We could show that AhR activity contributes to cytokine secretion. Most notably, TNF secretion is reduced in AhR−/− macrophages. TNF is known to induce NO which is important for parasite killing.

Thus, AhR is involved in Leishmania major induced effects in macrophages. We therefore analysed the possible effects of antagonizing AhR in C57Bl/6 mice *in vivo*: co-treatment with AhR antagonist CH-223191 during infection with L. major caused a reduction in skin transcript levels of Mip1-beta, IL1-beta, Cxcl2 and several other genes, while co-treatment with AhR agonist ITE upregulates the same cytokines in BALB/c mice. Our experiments show that AhR influences the expression of several important cytokines in the skin early after infection. Testing for *in vivo* relevance of AhR in Leishmania infection, we found that AhR−/− mice have a higher inflammatory reaction compared to WT, but no conversion of Th1 response.

We then treated susceptible BALB/c mice with degradable AhR ligand ITE on three consecutive days by local injection into the footpad and found a reduction in footpad swelling in the late phase of infection, showing that early manipulation of AhR signalling can influence disease progression in mice.

Pharmacology

P215

Mechanisms of fumaric acid ester action on granuloma formation

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Fumaric acid esters (FAEs) are a well established therapy option in psoriasis. In addition, several reports have described an effectiveness of FAEs on skin lesions in patients suffering from granulomatous diseases such as sarcoidosis, necrobiosis lipoidica and granuloma annulare. However, very little is known about the mechanisms involved. In this study, we investigated the influence of FAEs on giant cell formation; one of the hallmarks in the development of granuloma. Since giant cells originate from monocytes/macrophages we also wanted to know whether the FAEs dimethylfumarate (DMF) and monomethylfumarate (MMF) are able to influence oxidative stress levels and cell viability. Here, we made use of the human monocyte-like cell line U937 and primary human monocytes (pMo) isolated from peripheral blood of healthy donors via indirect magnetic bead labelling. Formation of multi-nucleated giant cells (MGC) was achieved after 72 h cultivation of primary human monocytes with conditioned medium obtained from concanavalin A-stimulated peripheral blood mononuclear cells. The fusion index was determined as the proportion of nuclei within MGC relative to the total number of cell nuclei as estimated by light microscopy. Cell viability and the induction of oxidative cell stress were investigated using flow cytometery techniques. We found that treatment of U937 or pMo cells with DMF for 72 h led to a significant decrease in cell viability at concentrations over 35 M (U937) and 21 M (pMo). In contrast, viability of both cell types remained over 80% with up to 140 M of MMF. In the presence of 7–21 M DMF, the number of MGC decreased significantly, and the fusion index was reduced by up to 50%. The effects of MMF were much less pronounced. Finally, the content of reduced glutathione in U927 and pMo cells was decreased by up to 80% during incubation with DMF but not with MMF, where no changes were seen even at concentrations up to 280 M. Hence it is likely that the therapeutic effects of FAEs in granulomatous diseases are mainly mediated via DMF-induced reduction of cellular antioxidants and a decreased monocyte cell viability, ultimately impacting the formation of MGC. Our findings contribute to a better understanding of the underlying effects of FAEs in the therapy of granulomatous diseases.

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The fumaric acid ester DMF promotes the intrinsic apoptotic pathway in human mast cells

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Mast cells are key effector cells in allergies and regulate autoimmune diseases such as psoriasis and multiple sclerosis. Fumaric acid esters are widely used for the treatment of psoriasis and have recently also been explored in multiple sclerosis. In the present study, we analyzed the apoptotic signaling of human mast cells in response to the fumaric acid ester dimethylfumarate (DMF). Apoptosis and viability of the human mast cell line HMC-1 and primary cord blood-derived mast cells (CBMC) was analyzed by flow cytometry. To study apoptotic signaling pathways, we performed immunoblotting and inhibition experiments. DMF was found to induce apoptosis in HMC-1 cells and CBMC in a dose- and time-dependent manner. DMF-mediated apoptosis of HMC-1 cells was associated with reduced expression of the anti-apoptotic proteins bcl-2 and bcl-xL and increased expression of the pro-apoptotic protein bax. Furthermore, DMF-induced apoptosis involved caspase-9, caspase-6 and cleavage of lamin A/C, which acts downstream of caspase-6. Interestingly, DMF also enhanced the sensitivity of CBMC towards TRAIL- and dexamethasone-induced apoptosis. Taken together, our data show that DMF induces apoptosis of human mast cells predominantly via the intrinsic apoptotic pathway and sensitizes mast cells towards other apoptotic stimuli. These findings may in part explain the beneficial effect of DMF in autoimmune diseases and provide a rationale for exploiting DMF in other diseases associated with mast cells.

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Comparison of non-cross-linked und cross-linked hyaluronic acid with regard to efficacy of the proliferative activity of cutaneous fibroblasts and keratinocytes *in vitro*

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Intradermal application of hyaluronic acid (HA) in varying chain length and crosslinking density is used routinely for hydrodynamic volume replacement of the extracellular matrix to reduce the clinical effects of ageing.

In vitro data show that via receptors of the hyaladherin group hyaluronic acid has additionally direct or indirect effects on cells. In the case of native non-cross-linked HA it has been proved that the proliferative and metabolic activity of cutaneous fibroblasts can be increased. The aim of the here presented studies was to investigate whether these effects can be proved also for cross-linked HA and how these effects can be quantified for different preparations.

The effect on proliferative activity in cultures of native cutaneous fibroblasts and keratinocytes was investigated for non-cross-linked HA, for non-cross-linked HA with added glycerol, for HA that was stabilized in the carboxyl and hydroxyl groups per inner esterification, and for HA that was chemically cross-linked by 1,4-butanediol-diglycidylether, mixed in small particles in a biphasic compound with native HA, each in different concentrations (0.1, 1.0 und 10.0 mg/mL).

HA that was stabilized in the carboxyl and hydroxyl groups per inner esterification induces the strongest proliferative effect on both cell types. Native non-cross-linked HA and chemically cross-linked HA shows a rather modest proliferative effect and on fibroblasts only, while non-cross-linked HA with added glycerol in high concentrations provokes a rather anti-proliferative effect.

The data show that HA does induce direct effects on cells depending on type and density of the cross-linkage. The practical relevance in terms of a metabolic filler effect needs to be verified in clinical studies.

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Cutaneous bioavailibility of dipeptides modulate by enhancer and colloidal carrier system

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Due to the lipophilic properties of the uppermost skin layer of the stratum corneum (SC) it is highly challenging to reach therapeutic concentrations of actives, especially hydrophilic drugs penetrate poorly. The purpose of this study was the improvement of the topical bioavailability of the hydrophilic dipeptides L-carnosine and its related compound *N*-acetyl-L-carnosine. Different strategies were investigated. On the one hand an enhancer molecule (1,2-pentylene glycol, PeG) was added to a standard preparation and on the other hand a microemulsion (ME) system was developed. Both were compared to the standard formulation without enhancer molecule. For all three preparations the penetration of the peptides in *ex vivo* human skin was investigated. This allows to make statements regarding dermal penetration, localization and distribution of the active substances in each skin layer as well as the influence of vehicle variations, in this case the addition of PeG or the incorporation of *N*-acetyl-L-carnosine in a ME system. For L-carnosine and *N*-acetyl-L-carnosine, the use of the standard preparation with PeG resulted in a significant increase of the substance within the SC. Approximately sixfold and higher dipeptide concentrations in the SC and the viable skin layers were detected at all experimental periods compared to the formulation without the enhancer molecule and the ME. High concentrations of the compounds were found after a short period of time in the viable skin layers after applying the enhancer molecule even in the concentrations of 5%. The application of the colloidal carrier system did not lead to a higher penetration rate of *N*-acetyl-L-carnosine in comparison to both standard preparations, although it must be said that the microstructure of the investigated ME might not have been optimal for the hydrophilic properties of the dipeptide.

Photobiology

P219

Clean hands save lives: photodynamic killing of human skin-related pathogens within seconds

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Introduction: In recent decades, the incidence of infections caused by multidrug-resistant Gram-positive and Gram-negative bacteria as well as Candida albicans has considerably increased not just in terms of morbidity and mortality, but also in terms of health care budgets and prolonging treatment times. At this point hand hygiene is one of the most important interventions for reducing transmission of nosocomial life-threatening microorganisms, like methicillin resistant *Staphylococcus aureus* (MRSA), enterohemorrhagic Escherichia coli (EHEC) or Candida albicans. All three pathogens have become a leading cause of infections in hospitals. Therefore successful inactivation, decolonisation or sterilization of pathogenic microorganisms is one important goal in a world of increasing multiresistant pathogens in fields like the food industry and medicine. The use of UV is dangerous and critical, because it can damage the DNA structure and induce mutagenesis. Chemical agents such as ethylene oxide or hydrogen peroxide for sterilization are limited because of their toxicity to eukaryotic cells. In clinical applications, a successful decolonisation of MRSA strongly depends on the compliance of the patients and the treatment protocol used. Especially EHEC is causing severe diarrhoea and, in a small percentage of cases, haemolytic-uremic syndrome (HUS) as reported for *E. coli* 104:H4 in Germany 2011. We revealed the possibility to inactivate very fast and efficiently MRSA, EHEC and *C. albicans* using the photodynamic approach. Various studies have shown that photodynamic process with light in the visible wavelength range and porphyrin-based photosensitizers,like TMPyP, exhibit significant photosensitizing activities against a broad range of pathogens only in the presence of light and oxygen. A disadvantage might be the long time treatments, which includes the incubation and irradiation procedure.

Aim of the study: By using a non-coherent light source and short incubation times of a few seconds, we aimed to achieve a fast and effective photodynamic inactivation of both bacteria and fungi yielding more than a 3 log10 ($\geq$99.9%) reduction.

Results: MRSA, EHEC and *C. albicans* were incubated *in vitro* with different concentrations of TMPyP for 10 s and illuminated with visible light [50 mW/cm^2] for 10 and 60 s. 1 μmol/l of TMPyP and an applied radiant exposure of 0.5 J cm^2 achieved a photodynamic killing of $\geq$99.9% of MRSA and EHEC. Incubation with higher concentrations (up to 100 μmol/l) of TMPyP caused bacteria killing of >5 log(10) ($\geq$99.999%) after illumination. Efficient Candida killing ($\geq$99.999%) was achieved first at a higher light dose of 12 J cm^2. Different rise and decay times of singlet oxygen luminescence signals could be detected in Candida cell suspensions for the first time, indicating different oxygen concentrations in the surrounding for the photosensitizer and singlet oxygen, respectively. This confirms that TMPyP is not only found in the water-dominated cell surrounding, but also within the *C. albicans* cells. Applying a water-ethanol solution of TMPyP on *ex vivo* porcine skin, fluorescence microscopy of histology showed that the photosensitizer was exclusively localized in the stratum corneum regardless of the incubation time.

Summary: TMPyP exhibited a fast and very effective killing rate of life-threatening pathogens within a couple of seconds that encourages further testing in an *in vivo* setting. Being fast and effective, antimicrobial photodynamic applications might become acceptable as a tool for hand hygiene procedures and also in other skin areas.

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UVA and UVB radiation changes endogenous molecules in different ways and influence therefore their ability to generate singlet oxygen – potential new pathways of cell damage?

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Solar UV radiation that reaches earth comprises UVA (320 EUR" 400 nm) and UVB (280 EUR" 320 nm) radiation. UV radiation is already known as initiator and promoter of carcinogenesis in skin. Cellular damage through UV radiation has been assigned either to indirect UVA damage via generated reactive oxygen species like singlet oxygen or to direct DNA damage caused by UVB radiation. Recently, it was shown that also UVB radiation is absorbed by endogenous molecules and can generate singlet oxygen.

In the present study, we aimed to investigate whether UVB or UVA radiation alters endogenous photosensitizers during irradiation and how this in turn may affect singlet oxygen generation.

Endogenous photosensitizers such as vitamins or unsaturated fatty acids were dissolved in ethanol solutions at different concentrations. Excitation of endogenous photosensitizers was performed at different wavelengths using a laser that was tunable in the UVB and UVA range. Photostability of the endogenous photosensitizers and hence singlet oxygen generation was investigated during irradiation. Once singlet oxygen was generated, it could oxidize the endogenous photosensitizers in solution. Subsequently, absorption coefficient remained either unaltered in the entire spectral range of UVB and UVA, increased or decreased. For example, when applying UVB-radiation with 6.6 J*cm-1, UVA absorption at 355 nm of FAD is unaltered, of Pyridoxine 5EUR(TM)phosphathydrate is decreased and of Arachidonic acid is increased. Likewise, the singlet oxygen generation of Pyridoxine 5EUR(TM) phosphathydrate and Arachidonic acid changed as well.

Our findings clearly show that experiments, in particular cell experiments *in vitro*, may yield different results when applying UVA or UVB alone, in parallel, or one after each other.

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Comparison of different new phenothiazine dyes and its antimicrobial activities

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Successful inactivation of pathogenic bacteria is one important goal in a world of increasing antibiotic resistant bacteria in medicine. Recently photodynamic inactivation of bacteria (PIB) has been shown very effective *in vitro*.
A common used singlet oxygen sensitizer is methylene blue. This photosensitizer is very well investigated and its singlet oxygen quantum yield is measured many times by indirect methods. Singlet oxygen generated by a given photosensitizer is a highly reactive oxygen species that is involved in various processes in biology and medicine, especially for inactivation of microorganisms. Using special IR photomultipliers, singlet oxygen can be directly detected in solutions by its extremely weak luminescence at 1270 nm.
The aim of the present study was to evaluate new derivatives of methylene blue an already known 'old school' photosensitizers to enhance the antimicrobial photodynamic efficacy.
Four phenothiazine derivatives were investigated by their physical and biological aspects. Therefore the singlet oxygen quantum yield of all sensitizers was measured directly at different concentrations and excitation wavelengths to estimate the effect of dimerization of the molecules. Also the effect of type I and type II mechanism for the inactivation of microorganism was investigated.
The photodynamic activity was investigated by toxicity test with *S. aureus* and *E. coli*. PIB resulted in a reduction of more than 3 log10 CFU (>99.9%) in bacteria suspension related to untreated controls. No reduction of bacteria CFU with PS or light alone was observed. Overall the new methylene blue derivatives showed under the present experimental conditions a pronounced antibacterial photodynamic effect against both bacteria species.
However these results should encourage the development of a specific side chain chemistry to enhance the photodynamic efficacy in the near future.

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UV exposure of tennis players

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Outdoor sport is part of leisure time behaviour in the western world which contributes significantly to the annual ultraviolet (UV) exposure. One of the most popular outdoor activities, both in adults and children is tennis. In our field study we measured UV exposure during a training match between two adults and - for the first time - the UV exposure of children playing tennis.
The players/parents of the players gave their consent to wear/that their children wear two electronic UV dosimeters (X 2000-10, Gigahertz Optics, Germany; UVA and UVB/C sensor, temperature, 1 min measuring interval), one on the forehead fixed to a shielded headband, the other fixed to the calf by an elastic band with Velcro fastening. We calibrated the dosimeters prior to the field test. The training match between the adult test persons A (43 years) and B (51 years) took place on June 30th from 1:00 to 2:36 PM, the two adolescents C and D, both 17 years of age, played on August 23rd between 1:45 and 14:52 PM on an almost north-south directed tennis court in Vienna. The players were observed by two members of the study team. One of the observers took photos of the players and at 15 min interval standardized photos of the sky. We measured ambient radiation with the help of two dosimeters fixed to a tripod in horizontal position: one dosimeter with the UV sensors directed upward, the second dosimeter with the sensors directed to the tennis ground in order to measure the reflectance. Sky was absolutely clear on June 30th with an ambient temperature of 35°C, whereas on August 26th there was variable cloudiness. The temperature on that day was around 30°C. The test persons were asked to behave as usual especially with respect to physical activity and photo protective measures. All our test persons wore similar tennis kits: T-shirt, shorts or short skirt, and tennis socks. All of them applied sunscreens with SPF 30. Three of them used sunglasses.
The UV exposures (standard erythema dose = SED) on June 30th were: test person A (head 2.82/calf 2.58), B (head 5.09/calf 1.41) which represent 20.05% to 72.3% of ambient radiation (7.05 SED). The respective values for the training match on August 26th were: test person C (1.54/0.63), test person D (1.98/0.62) which is equivalent to 32% to 100.15% of ambient (1.98 SED). 2.6% of the ambient radiation was reflected from the ground (red sand).
The adult tennis players played two sets, our adolescent test persons, however, played only one set. Children are at high risk especially when playing in spring and early in summer when ambient radiation is highest. This should be considered by organizers of tennis tournaments and tennis trainers when scheduling their training lessons.

Pruritus

P223

The expression of the neurokinin 1 receptor (NK1R) in atopic dermatitis and prurigo nodularis

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Chronic pruritus induces a high burden in patients and still constitutes a great therapeutic challenge. The neurokinin 1 receptor (NK1R) has been recently discussed as a potential candidate for the antipruritic treatment. Its endogenic mediator, the neuropeptide Substance P (SP) is deemed to be a relevant mast cell activator in pruritic diseases and is speculated to be a key mediator for itching in several skin diseases, such as atopic dermatitis (AD) and prurigo nodularis (PN). The NK1R is expressed in various cell types in the skin, such as keratinocytes, mast cells and fibroblasts. The aim of the present study was to determine the expression of the NK1R in the lesional, pruritic skin of patients with AD ($n = 13$) and PN ($n = 13$) compared to healthy controls ($n = 10$) using different methods (immunofluorescence, quantitative Real Time-PCR and Western Blot). The studied group consisted of women in an age range of 22–77 years (mean age AD 41 years, PN 66 years and healthy controls 48 years). The NK1R was found in all patients mainly intercellular in the epidermis by immunofluorescence staining. Compared to normal skin of healthy controls, a more distinct signal of NK1R was observed in pruritic patients. With Real Time-PCR analysis, we found a significant down-regulation of the NK1R RNA level in AD and PN compared to healthy controls, with no differences between the two patient groups. On protein level we detected various isoforms of the NK1R in all samples. The variants of the NK1R at 75 and 30 kDa are basically found in both groups of patients and also in healthy controls, whereas in patients with PN the truncated variant of the receptor seems to be more distinct. In the skin of patients with PN which have been treated by the NK1R antagonist aprepitant for 4 weeks, an intercellular epidermal up-regulation of NK1R in immunofluorescence stainings was found. This is the first investigation on the expression of the NK1R in AD and PN including an analysis before and after a therapy with a NK1R antagonist. In sum, the results show a down-regulation of the receptor on RNA level but an up-regulation during antipruritic therapy in patients with PN using a specific antagonist. These results argue again for NK1R being an interesting target in pruritic diseases. Moreover, the antipruritic effect is currently debated to be related to binding of aprepitant in peripheral tissues or the central nervous system. Our study argues for at least a cutaneous reconstitution of the receptor expression during aprepitant therapy. Further investigations have to enlighten the particular role of epidermal NK1R in pruritic skin diseases.

P224

Increased VEGF expression and enhanced vascular remodelling in the skin of patients with prurigo

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Prurigo is a very difficult to treat condition which is characterized by severe chronic pruritus presenting with chronic secondary scratch lesions. The pathomechanisms underlying chronic itch in prurigo are still largely unknown, and to date no efficient targeted therapy is available. It has recently been speculated that the activation of STAT3 in the skin of patients with prurigo is at least in part responsible for the observed symptoms. Vascular endothelial growth factor (VEGF) is a known activator of STAT3 and epidermal keratinocytes can be efficient producers of VEGF. Therefore, we aimed to identify and characterize the role of VEGF in the pathogenesis of prurigo. First, we assessed serum levels of VEGF and detected significantly higher VEGF concentrations in patients with prurigo as compared to healthy controls (191.938.5 vs 48.78.4 pg/ml, $P < 0.001$). Furthermore, in prurigo patients, levels of VEGF correlated with the disease activity ($r = 0.525$, $P < 0.005$). It has been shown previously that VEGF is constitutively expressed at low levels in normal epidermal keratinocytes, and we speculated that the increased serum levels of VEGF are due to an increased production and release of VEGF from epidermal keratinocytes. Thus, we next performed immunohistochemical analyses of lesional skin from prurigo patients as well as from skin of healthy controls. The stainings revealed an increased immunoreactivity for VEGF not only in the epidermis but also in the dermis and subcutis of prurigo patients while VEGF receptor expression was comparable to healthy controls. As VEGF is an important factor in vascular remodelling, we next analyzed the number and size of vessels in the skin of prurigo patients and healthy controls. To this end, we stained lesional skin of prurigo patients and of healthy controls with an antibody against CD31, a marker for endothelial cells. Here, we detected a strong increase in the number of blood vessels in prurigo as compared to controls (13.50.2 vs 5.60.6, per microscopic field, $P < 0.05$). The number of vessels detected in the skin of patients correlated closely with the epidermal thickness in prurigo lesions ($r^2 = 0.86$, $P < 0.0001$). Taken together, we show that VEGF is strongly upregulated in the skin of patients with prurigo nodularis and that VEGF serum levels are associated with higher disease activity. Furthermore, we describe a profound vascular remodelling in patients with prurigo. Whether VEGF contributes to the itch intensity in the patients and by which mechanisms VEGF can contribute to the disease is still unknown and requires further investigations.

Tumor Biology

P225

Dimethylfumarate suppresses prostate cancer cell proliferation and fortifies chemotherapeutic action

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Prostate cancer is the most common tumor entity in male patients and therefore is an important medical problem. In contrast to localized forms of prostate carcinoma, the metastatic disease is still a challenge for the oncologists and the treatement options are limited.
Recent evidence suggests, that Dimethylfumarate (DMF), known as a highly potent anti-psoriatic agent, might have anti-tumorigenic properties. There are limited data demonstrating that DMF induces apoptosis or enhances the effects of radiation treatment in head and neck cancer. In addition, the anti-angiogenic characteristics of DMF might bolster its anti-tumorigenic actions.
To analyze the effects of DMF on prostate carcinoma cell lines, we first performed cytotoxicity assays with the androgen dependent cell line LNCAP and the androgen independent cell line PC-3. Up to 100 M DMF no LDH release could be demonstrated. In further analysis we could show, that DMF suppresses prostate carcinoma cell proliferation in a concentration dependent manner. These effect could be paralleled with reduced prostate specific antigen (PSA) expression. In functional tumor invasion assays we could demonstrate that DMF treatment reduces prostate cancer cell invasion almost as effective as the first-line chemotherapeutic Docetaxel. To examine whether these effects are conveyed by apoptotic mechanisms we studied the amount of apoptotic nucleosomes by ELISA-analysis. There was no significant apoptosis induced by DMF in LNCAP as well as PC-3. Therefore, we performed cell cycle analysis. Interestingly, DMF induced an G0/G1 arrest in both prostate carcinoma cell lines. To examine the underlying mechanisms of the cell cyle arrest, we analyzed the expression profile of important cell cyle regulator proteins as for example p53, p21, p27, Cyclin D, CDK 4,6. Interestingly, in LNCAP DMF induced p53, p21 and p27 whereas in PC-3, which harbors a p53 mutation, only p21 and p27 were induced. The other cell cycle regulators were not influenced by DMF treatment.
In further experiments, possible additive effects of DMF treatment combined with the first-line chemotherapeutic Docetaxel, were evaluated. Here, it could be demonstrated, that the combination of both agents is much more effective than the chemotherapeutic agent alone.
These data provide the first evidence, that DMF inhibits prostate cancer proliferation by reinduction of important cell cycle inhibitors leading to G0/G1 cell cycle arrest. In addition, the combination of the first-line chemotherapeutic Docetaxel and DMF provides additive anti-cancer effects. Hence, DMF might be an interesting agent in the treatment of prostate cancer and is worth for further *in vivo* analysis.

P226 (O07)

miR-638 as an oncomiR in malignant melanoma

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Malignant melanoma is a cancer of the pigment producing cells of the skin which is highly aggressive and often treatment-resistant in its metastatic stage. The role of microRNAs (miRNAs) both as oncogenes and tumor suppressors has been widely accepted. In this study, we screened for the expression of 670 miRNAs in a cohort of primary and metastatic melanoma patient samples using miRNA Taqman Low density arrays. We identified miR-638, which had significantly higher expression in lymph node and distant cutaneous metastases as compared with primary melanomas. Moreover, there was no expression of miR-638 in freshly isolated primary melanocytes which suggested that miR-638 might play an important role both in initial cellular transformation and metastatic progression. Expression of miR-638 directly correlated with the increasing tumor thickness of primary tumors which further substantiated our findings. Overexpression of miR-638 significantly increased proliferation of melanoma cells both under normal growth conditions and upon low density seeding. Furthermore, miR-638 overexpression enhanced cell migration and improved anchorage-independent growth of melanoma cells. These results suggested that miR-638 is indeed an oncogenic miRNA (oncomiR) in malignant melanoma. In line with this, a number of tumor suppressors including TFAP2B, PRDM2, HIC2 and HOXB13 have been predicted to be targeted by miR-638. Interestingly, miR-638 is encoded at the intronic regions of a potential oncogene DNM2 (Dynamin 2). We found a positive correlation between the expression patterns of miR-638 and DNM2 in melanoma cell lines suggesting that both can cooperate in enhancing the tumorigenic potential of melanoma cells. Indeed, knockdown of miR-638 and DNM2 reduced migration of melanoma cells *in vitro*. Taken together, our findings demonstrate that miR-638 is overexpressed during melanoma progression and enhances the oncogenic and metastatic properties of melanoma cells. In conclusion, targeting miR-638 might be a therapeutic possibility for malignant melanoma.

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Targeted hydroxyethyl starch (HES) nanocapsules for immunotherapeutic approaches

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Engineered nanocapsules (NC) carrying drugs emerged as a promising strategy to tune the immune system or to target the tumor itself. Based on the ability to modify the surface of the particles and to incorporate distinct drugs, NC can be tailored for various applications in medicine and biology. In the present study, NC composed of the natural biomolecule hydroxyethyl starch were successfully generated using the inverse miniemulsion process. This technique allows the formation of stable NC whose properties can be tailored individually according to specific requirements. All fluorescently-labeled NC revealed a size of about 200 nm as measured by dynamic light scattering. After NC synthesis the surface was functionalized with DBCO (dibenzylcyclooctyne-NHS ester) groups that were used for covalent linkages in a 'click-chemistry'. For this purpose, NC-targeting proteins were azide-functionalized via NHS-ester chemistry. The two different proteins, the cytokine IL-2 (HES-IL-2 NC) and the anti-DEC205 antibody (HES-DEC205 NC) were chosen to target CD4+CD25high (regulatory) T cells and immature dendritic cells (iDC), respectively. Following NC functionalization with IL-2 or anti-DEC205 antibody the functional groups on the capsule surface are quantified and the biological activity of the cytokine and antibody was verified afterwards via proliferation assays (IL-2) and immunoblotting (antibody binding and specificity for DEC205). Proliferation assays with the IL-2-dependent CTLL-2 cells verified that IL-2 bound to the NC surface was still biologically active after click chemistry. In addition, CD4+CD25high activated T cells stained with a proliferation dye revealed a strong proliferation when incubated with HES-IL-2 NC compared to control HES NC as assessed via flow cytometry. Increased CTLL-2 and CD4+CD25high activated T cell proliferation after HES-IL-2 NC treatment already indicates NC uptake via IL-2 receptor interaction and internalization. Anti-DEC205 antibody functionalized HES NC revealed an increased uptake by bone marrow-derived mouse iDC compared to DBCO-functionalized HES NC. HES-DEC205 NC uptake by mouse iDC was dose-and time-dependent.

The fundamental progress in the development of engineered nano- and microparticles as drug delivery systems offers enormous means for treatment of cancer and other diseases. Especially the fact that the targeting molecules on the particle surface as well as the drugs inside are interchangeable in most cases renders biodegradable nanoparticles very attractive to interfere with or regulate immune responses against carcinomas.

P228

PKCβ pathway is a new candidate for targeted therapy in malignant melanoma

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Malignant melanoma is a highly aggressive tumor with increasing incidence and high mortality rates in the metastatic stage. The molecular mechanisms underlying initial tumor development and further progression are still poorly understood. In the present study, we tried to identify new intracellular signalling pathways or networks important for melanoma cell growth, which might later serve as targets for therapeutic intervention. A large-scale loss-of-function screen was performed for eight melanoma cell lines using a genome-wide lentiviral RNAi library. Seventy eight signalling kinases were identified, targeted by at least two siRNAs. A number of these were validated and showed antiproliferative effects on melanoma cell lines after knock-down. In further analyses, the bioinformatic tool STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) and the Ingenuity pathway analysis were used to identify particular pathways and signalling networks. ERK2, MEK2, JNK1/2 and protein kinase C beta (PKCβ) were identified as top candidates within signalling networks. Since ERK2, MEK2, JNK1/2 have been intensively studied in many reports we focussed on newly identified PKCβ. PKCβ phosphorylates and activates tyrosinase and is bound to the melanosome by the receptor for activated C-kinase-I (RACK-I). Real-time PCR analysis showed high expression of PKCβ in lymph node metastases compared with primary melanomas suggestive for a role for PKCβ in early melanoma metastasis. Interestingly, cutaneous metastases had lower expression levels than lymph node metastases and primary tumors. These findings were further functionally validated in melanoma cells with transient and stable knockdown of PKCβ using a lentiviral system. Downregulation of PKCβ indeed led to reduction of clonogenecity, proliferation and migratory capacity of melanoma cells. *In vivo* experiments further substantiated the *in vitro* results. PKCβ should therefore be considered as a potential target for the treatment of metastatic melanoma. Small molecule inhibitors for PKCβ have already been tested in clinical trials for other tumor entities.

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Targeting hyperactivation of the AKT survival pathway to overcome therapy resistance of melanoma brain metastases

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Brain metastases are the most common cause of death in patients with metastatic melanoma, and the RAF-MEK-ERK and PI3K-AKT-mTOR signaling pathways are key players in melanoma progression and drug resistance. The BRAF inhibitor vemurafenib significantly improved overall survival. However, brain metastases still limit the effectiveness of this therapy. In a series of patients, we observed that treatment with vemurafenib resulted in substantial regression of extracerebral metastases, but brain metastases appeared or progressed. This study aimed to identify factors that contribute to treatment resistance in brain metastases.

Matched brain and extracerebral metastases from melanoma patients had identical ERK, p-ERK, and AKT immunohistochemistry staining patterns, but there was hyperactivation of AKT in the brain metastases as detected by p-AKT staining. Mutation analysis revealed no differences in BRAF, NRAS, or KIT mutation status in matched brain and extracerebral metastases. In contrast, ERK, p-ERK, AKT, and p-AKT expression was identical in monolayer cultures derived from melanoma brain and extracerebral metastases. Furthermore, melanoma cells stimulated by astrocyte-conditioned medium showed higher AKT activation than melanoma cells stimulated by fibroblast-conditioned medium. Inhibition of PI3K-AKT signaling resensitized melanoma cells isolated from a vemurafenib-resistant brain metastasis to vemurafenib.

Brain-derived factors appear to induce hyperactivation of the AKT survival pathway and to promote the survival and drug resistance of melanoma cells in the brain. Thus, inhibition of PI3K-AKT signaling shows potential for enhancing and/or prolonging the antitumor effect of BRAF inhibitors or other anticancer agents in melanoma brain metastases.

P230 (O13)

Yes-associated protein 1 (YAP1) promotes melanoma metastasis

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Melanoma is a highly aggressive neoplasm that metastasizes early during progression. The genetic basis of melanoma invasion and metastasis is only partially understood. Here we show recurrent activation of the Hippo pathway as a mechanism promoting metastatic spread of melanoma. We demonstrate focused amplifications of Yes-associated protein 1 (YAP1), the upstream kinase PAK1, and focused deletions of its negative regulators NF2 and LATS1 in 34.5% of melanomas. YAP1 protein, the transcription cofactor acting downstream in the Hippo pathway, is highly expressed in 56% of thick (? 2 mm) primary melanomas, and YAP1 levels are associated with decreased overall survival ($P = 0.013$). *In vitro*, YAP1 overexpression promotes the invasive potential of human melanoma cells, while YAP1 knock-down significantly suppresses their invasiveness. *In vivo*, YAP1 overexpression strongly promotes melanoma metastasis formation in a murine xenograft model. Our study identifies the Hippo pathway as an oncogenic driver for the metastatic phenotype of melanoma.

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6- and 8-prenylnaringenin, novel natural histone deacetylase inhibitors found in hops, exert antitumor activity on melanoma cells

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We report a novel mode of action of 6- and 8-prenylnaringenin (PN) on melanoma cells: Inhibition of cellular histone deacetylases (HDACs).

In silico, 6-PN and 8-PN fit into the binding pocket of HDAC2, 4, 7 and 8, binding to the zinc ion of the catalytic center essential for enzymatic activity. *In vitro*, 100 M of 6-PN or 8-PN inhibited all conserved human HDACs, induced hyperacetylation of histone complex H3 in SK-MEL-28 and BLM melanoma cells and decreased proliferation of SK-MEL-28 and BLM melanoma cells, HT-29 colon and MCF-7 breast cancer cells. An *in vivo* chicken embryotoxicity assay revealed little toxicity. In epidermal skin reconstructs both PNs abrogated proliferation and invasion of BLM cells. This effect was apoptosis-independent, accompanied by down-regulation of mTOR-specific pS6 protein and induction of autophagy, as determined by a shift from LC3-I to LC3-II.

Our data suggest that 6-PN and 8-PN might be useful as natural nutritions for melanoma prevention or as epigenetically-active molecules for melanoma therapy.

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Neuropilin-1 deficiency on CD4+Foxp3+ regulatory T cells impaires tumor growth in melanoma

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High numbers of tumor-infiltrating Foxp3+ regulatory T cells (Tregs) are considered as a marker for metastasis and are associated with poor overall survival among patients with certain types of malignant neoplasms, including melanoma. Tregs within the tumor microenvironment supposedly suppress local antitumor immune responses, although, the precise mechanism by which Tregs infiltrate the tumor is still unclear.

We provide evidence that Neuropilin-1 (Nrp-1), highly expressed by Foxp3+ Tregs, regulates the immunological antitumor control by guiding Tregs into the tumor in response to tumor-derived Vascular Endothelial Growth Factor (VEGF). T cell-specific ablation of Nrp-1 expression results in a significant breakdown in tumor immune-escape in various transplantation models and in the spontaneous, endogenously-driven melanoma model (MT/ret) associated with strongly reduced tumor growth and prolonged tumor-free survival. Strikingly, numbers of tumor-infiltrating Foxp3+ Tregs were significantly reduced accompanied by enhanced activation of CD8+ T cells within tumors of T cell-specific Nrp-1-deficient mice. Additionally, this phenotype can be reversed by adoptive transfer of Nrp-1+ Tregs from wildtype mice. Thus, the data strongly suggest that Nrp-1 acts as a key mediator of Foxp3+ Treg infiltration into the tumor site resulting in a dampened anti-tumor immune response and enhanced tumor progression.

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Loss of Noxa and Puma have inverse effects on melanomagenesis in a predisposed, genetically-defined mouse melanoma model

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Studies to evaluate the contribution of the intrinsic apoptosis pathway to melanoma biology and drug resistance are often compromised by the *in vitro* culture systems and xenograft models utilised. While such models have their advantages, adaptation of cells to culture *in vitro* and subsequent engraftment risks further selection for defective apoptosis. Furthermore, some important aspects are missing *in vitro*, as cells are not grown in their natural environment or with an intact immune system. Genetically defined, predisposed mouse models obviate each of these problems. In this project, an established mouse model of melanoma (Cdkn2a −/−, Tyr-HRAS) was utilised, which mimics lesions common in human melanoma. The BH3-only proteins Noxa and Puma were chosen as appropriate exemplars for intervention in the apoptosis pathway as there is evidence supporting their involvement in melanomagenesis and progression. The melanoma model mice were thus crossed with either Puma −/− or Noxa −/− lines. These models were used to investigate the effect of loss of Puma or Noxa on melanoma biology, and its resistance to treatment. Mice of all three cohorts developed melanomas and other tumours. Loss of Noxa delayed melanomagenesis, decreased tumour penetrance and number of

melanomas but – once tumours were established – accelerated melanoma growth compared to the control and Puma −/− cohorts. We speculate that loss of Noxa may be rescued by induction of other BH3-only pro-apoptotic proteins e.g. Puma, which could be responsible for the delayed melanomagenesis. Preliminary data from experiments addressing this question are discussed.

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Cancer cells and galaxies – novel image analysis techniques identify subcompartment-specific cell cycle dynamics and motility in melanoma

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The proliferative/invasive capacity of melanoma cells may be regulated by the local microenvironment and gene expression changes. We aimed to determine the effect of the tumor microenvironment on cell cycle dynamics and motility of individual melanoma cells. We developed a model to visualize melanoma cell cycle dynamics in real-time *in vitro* and *in vivo*. Cells expressing the fluorescence ubiquitination cell cycle indicator (FUCCI) system appear red in G1, yellow in S and green in S/G2/M-phase. FUCCI-melanoma cells were grown as organotypic 3D-spheroids and implanted into a collagen matrix to mimic tumor architecture and microenvironment, or as xenografts in NOD/SCID mice.

We analyzed the distribution and motility of cycling cells in spheroids using Volocity software and custom tracking software. Cells at the periphery of the spheroids were cycling and highly motile, invading into the surrounding collagen, while the slow moving cells in the interior of the spheroid were arrested in G1, presumably due to decreased access to oxygen and nutrients. This proliferating 'ring' was also observed at the periphery of tumor xenografts derived from some, but not all cell lines. Notably, tumors without a proliferating ring were derived from cells expressing high levels of MITF. Although MITF-knockdown did not fully restore the proliferating ring in tumors, the phenotype was partially rescued. Applying a spatial analysis technique used in astrophysics, we show that MITF-knockdown tumors have increased clustering of cells in S/G2/M-phase (patches of proliferation), while control MITFhi tumors have a more random distribution of cycling cells.

Our data suggest that both the microenvironment and MITF expression are able to regulate the subcompartment-specific distribution of differentially cycling cells in melanoma. This may impact the differential sensitivity of cells to melanoma therapy.

P235 (O23)

Regulation of the transcription factor c- Jun in malignant melanoma by the cytoskeleton

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Malignant melanoma is an aggressive tumor derived from melanocytes. Recent studies have shown that c-Jun (member of the AP-1 transcription factor family) plays an important role in this disease. A deregulation of transcriptional activity is often found during tumor development and the constitutive activity of the AP-1 transcription factor family effects the expression of a variety of regulators involved in melanoma development and metastasis.

In previous studies we determined that c-Jun is regulated at the post-transcriptional level in melanoma. Here, the cytoskeletal network seems to play an important role in c-Jun regulation and thus AP-1 activation (Spangler et al., 2011), however, the precise role and the interaction with c-Jun stayed elusive. Results of AP-1 luciferase reporter gene assays after Taxol and Nocodazol treatment suggested that the microtubule dynamics, and not the actin dynamics, are important for AP-1 activity. Moreover, decreased AP-1-DNA binding after Taxol and increased AP 1 DNA binding after Nocodazole treatment in melanoma cell lines could be detected by electrophoretic mobility shift assays. Due to the influence of microtubule disrupting agents (MTDs) on AP-1 activity we speculated c-Jun to be regulated by microtubule dynamics. Therefore, we performed transfection experiments of Hmb2–5 cells, a model system resembling melanocytes lacking c-Jun expression, with a c-Jun expression plasmid (HAJun MUT1; Kappelmann et al., 2012). We then analyzed the c-Jun induced AP-1 activity by AP-1 luciferase reporter gene assays and detected identical effects after MTD treatment as mentioned above. These results denote that the regulation of AP-1 activity via microtubule is c-Jun specific. To find out the effects of Taxol and Nocodazole treatment on c-Jun protein we performed western blot analysis against c-Jun after MTD treatment and observed that the cytoskeletal network seems to play a role in stabilizing c-Jun protein. Stabilization of microtubule by Taxol treatment resulted in a decreased level of c-Jun protein in the nucleus whereas Nocodazole treatment accordingly led to a nuclear c-Jun accumulation. Furthermore, we demonstrated for the first time a direct interaction between tubulin and c-Jun. Here, we performed microtubule spin down assays, which showed no binding activity of c-Jun to polymerized microtubule. Hence, we speculated if c-Jun protein might interact with monomeric tubulin. Co-immunoprecipitation of alpha-tubulin and c-Jun respectively from total melanoma cell lysates showed the presence of c-Jun in alpha-tubulin precipitation and of alpha-tubulin in c-Jun precipitation.

In summary, we could observe that microtubule dynamics significantly influence AP-1 activity in melanoma cells. Moreover, we could show that this influence on AP-1 activity by MTDs is c-Jun specific. Furthermore, we demonstrated for the first time a direct interaction between the transcription factor c-Jun and alpha-tubulin, which seems to stabilize c-Jun protein expression and thus AP-1 activity. Taken together these findings could possibly elucidate new regulatory mechanisms of c-Jun protein in malignant melanoma and thus lead to the identification of novel therapeutical targets.

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Modulation of melanoma cell functions by RGD presented on tunable nano-structered surfaces

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Functional regulation of adhesion receptors such as the $\alpha v\beta 3$ integrin crucially influences the aggressiveness of melanoma and other tumor cells. By recognizing the tripeptide sequence RGD, integrins mediate essential cellular functions such as adhesion, migration and proliferation. This is why the interaction of integrins with the RGD-binding motif has become a target of therapeutic approaches. Curiously enough, a very recent study showed that low doses of RGD analogues paradoxically stimulated tumor growth and angiogenesis. Indeed, in some cases blockage of specific ligands can lead to unforeseen and even adverse effects apparently depending on the number of blocked receptors. For an in-depth investigation of such molecular interactions we need new techniques using highly defined conditions on the single-molecule level.

We have established and characterized a highly tunable *in vitro* model system in which an $\alpha v\beta 3$-specific RGD is presented at defined distributions and densities on the molecular level. Toward this end, glass substrates were structured with gold particles using block copolymer nanolithography. The distance between gold nanoparticles can be adjusted between 25 and 300 nm. In contrast to conventional systems, our new system allows precise tuning of receptor densities in a physiologically relevant range as well as proper polarization of the receptors.

Using this method, we have shown for the first time that $\alpha v\beta 3$-expressing melanoma cells spread and move best on an 'intermediate' ligand density with an interparticle spacing of 60 nm. A more as well as a less dense presentation of RGD conspicuously attenuated tumor cell spreading. Further immunofluorescence investigations of melanoma cells bound to different site densities of RGD revealed significant differences in the formation and distribution of focal adhesions as well as the cytoskeleton. Blocking peptides at suboptimal concentrations appeared to 'shift' the optimal ligand densities.

In conclusion, we propose that molecularly defined cell-matrix interactions determine the 'optimal' conditions for tumor progression. The model system presented here mirrors crucial aspects of such regulations in an individually tunable fashion. Given that less-than-optimal dosages of anti-tumoral drugs may shift the adhesiveness of a given tumor cell population toward a 'permissive' receptor-ligand ratio, our results are an important step to tailor personalized diagnostics and therapies.

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Do Merkel cell polyomavirus positive Merkel cell carcinoma cells require expression of the viral small T antigen?

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Increasing evidence suggests that Merkel cell carcinoma (MCC) is often caused by the Merkel cell polyoma virus (MCV). The viral sequences integrated in the MCC genome encode for two potential oncoproteins, i.e. the small T antigen (sT) and a truncated Large T antigen (LT). Indeed, sT has recently been shown to bear transforming activity. A different, but with respect to therapeutic implications more important question is whether established MCC cells require sT for growth and survival. Here we provide evidence suggesting that this is not the case. Although, application of two different sT specific shRNAs lead to variable degrees of growth retardation in MCV-positive MCC cell lines these effects appear not to be sT-specific since growth of MCV-negative cell lines is also affected by these sT shRNAs, and ectopic expression of shRNA-insensitive sT does not revert the growth inhibition. Moreover, the unambiguous and specific growth retardation induced by application of an shRNA targeting both T antigens, can be completely rescued by ectopic expression of only LT sustaining a dispensable role of sT. Altogether, our results suggest that targeting MCV LT is the more promising approach for future therapies of MCC than targeting MCV sT.

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Hdm-2 but not the Merkel cell polyoma virus T antigens keep the p53 pathway in check in p53 wild type Merkel cell carcinoma cells

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Merkel cell carcinoma (MCC) is a rare but very aggressive skin cancer with viral etiology. The respective Merkel cell polyoma virus (MCV) belongs to a group of tumor viruses that encode the so called T antigens (TA) which can induce tumorigenesis by interfering with cellular tumor suppressor proteins like p53. To explore possible modes of p53 inactivation in MCC we characterize a set of MCC cell lines by p53 sequencing, p53 expression analysis and by applying a p53 reporter gene assay. In the majority of MCC cell lines p53 is genetically wild type, is expressed and displays some transcriptional activity, which, however, is not sufficient to effectively restrict cellular survival or growth. But the MCV TAs are not responsible for this critical lack in p53 activity as TA knockdown by shRNA in MCV-positive MCC cells does not induce p53 activity. In contrast, inhibition of the ubiquitin ligase HDM-2 by Nutlin-3a leads to p53 activation and to p53 dependent apoptosis or cell cycle arrest in five out of seven p53 wild type MCC cell lines. Our results demonstrate the feasibility of p53 reactivation in MCC and highlight p53 as a potential target for future therapies for this deadly disease.

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Tlr4-dependent inflammation in the tumor microenvironment expands myeloid-derived suppressor cells that promote metastatic progression of melanoma in the Hgf-Cdk4 mouse model

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A hallmark of chronic inflammation in the tumor microenvironment is the systemic expansion of myeloid-derived suppressor cells (MDSCs). These cells not only downregulate cellular immune responses but also facilitate invasive growth and metastatic spread of tumor cells. Here we experimentally investigated the impact of TPA-induced chronic skin inflammation on the development of MDSCs and the growth of melanoma in the Hgf-Cdk4 mouse model. Repetitive epicutaneous applications of TPA effectively stimulated epidermal hyperplasia, skin immune cell infiltration and systemic expansion of MDSCs in healthy wildtype mice. Continuous TPA treatment of carcinogen-exposed Hgf-Cdk4 mice increased the number of tumor-induced MDSCs and selectively enhanced the infiltrative and metastatic growth of primary cutaneous Hgf-Cdk4 melanoma cells. The impact of TPA-induced inflammation on MDSC expansion and tumor progression could be reproduced in serial transplantion experiments with a highly metastatic Hgf-Cdk4 melanoma cell line. Importantly, antibody-mediated transient depletion of MDSCs decreased the growth of spontaneous lung metastases. Furthermore, TPA-induced inflammation, MDSC expansion and promotion of melanoma metastases was completely absent in Tlr4-deficient mice. Taken together, these results show that chronic TPA-induced inflammation in the microenvironment of cutaneous melanomas leads to systemic MDSC expansion which promote metastatic tumor progression in a Tlr4-dependent manner.

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Effective melanoma immunotherapy with cytosolically targeted pIC requires type I IFN-dependent activation of NK cells by myeloid immune cells

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Activation of the ubiquitously expressed cytosolic RNA recognition receptors with immunostimulatory RNA (isRNA) emerges as a new treatment option for cancer. This mimics a viral infection and induces a strong type I interferon response. Here we investigated the cell type specific role of type I IFNs for the anti-tumor efficacy of the prototypical isRNA polyI:C using Ifnar1 competent and deficient melanoma cell lines established from Hgf-Cdk4R24C mice in combination with global and tissue-specific Ifnar1 knockout mice. We found that cytosolically targeted pIC complexed with polyethylenimine (PEI-pIC) more effectively impaired growth of primary melanomas than naked pIC. This was accompanied by increased immune cell infiltrates and more abundant necrosis. The anti-tumor effects of both PEI-pIC and naked pIC were abrogated in Ifnar1-deficient Hgf-Cdk4R24C mice. Treatment of melanoma cells with cytosolically targeted pIC promoted IFNa production, chemokine secretion and apoptosis in Ifnar1-competent and Ifnar1-deficient Hgf-Cdk4R24C mouse melanoma cells *in vitro*. However, tumor transplantation experiments showed that effective melanoma therapy with cytosolically targeted pIC strictly depended on a functional type I IFN system on host but not tumor cells *in vivo*. Conditional deletion of the Ifnar1 gene specifically in LysM+ or CD11c+ myeloid

cells also largely abrogated the therapeutic efficacy of PEI-pIC. Furthermore, Ifnar1-competent but not Ifnar1-deficient myeloid immune cells activated Ifn-γ production in NK cells and antibody-mediated elimination of NK cells or blockade of Ifn-γ strongly reduced treatment efficacy. Taken together, these data indicate that type I IFN-dependent activation of NK cells by myeloid immune cells is critically required for effective melanoma immunotherapy with cytosolically targeted isRNA.

P241

Mast cell derived mediator chymase as a potent regulator of uPA/ uPAR system in cutaneous tumors

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Reorganisations of the cytoskeleton of tumor cells, adherence to adjacent cells and to the surrounding extracellular matrix (ECM) and degradation of ECM are cellular processes which determine tumor invasion and metastasis. The urokinase (uPA) mediated plasminogen activation system is involved in all these events. The central molecule of this system, the uPA receptor (uPAR), is able to regulate the proteolytic activity of its ligand on the cell surface and the integrin mediated cell adhesion to ECM. Several immunohistochemical studies indicated that mast cells (MC) are present in increased number in tumor-stroma and may be involved in the regulation of tumor progression. In this context, we were interested to study, how mast cells regulate the uPA/uPAR system in cutaneous tumors.

Cutaneous tumor cell lines derived from melanoma or squameous cell carcinoma (SCC) were cultivated for 24 h with or without conditioned medium derived from IgE-activated or non-activated primary dermal mast cells. Protein expression of uPA and uPAR was estimated by ELISA in supernatants of each individual cell line.

SCC cells constitutively released uPA (SCC-12 10 ng/ml, SCC-13 20 ng/ml). However stimulation with conditioned medium inhibited the release of uPA. Pre-incubation of the supernatants with chymase inhibitor SBTI but not with tryptase inhibitor aprotinin restored the level of uPA to control levels. Stimulation of SCC and melanoma cell lines with conditioned medium led to a strong and significant increase in uPAR release (in SCC-12 cells from 50 to 1700 pg/ml, in SCC-13 from 50 to3800 pg/ml in mel-5 from 800 to 2500 pg/ml and in mel-6 from 500 to 2000 pg/ml), which was significantly attenuated by the chymase inhibitor SBTI but not of the tryptase inhibitor aprotinin. This result suggests that mast cell derived mediators, in particular chymase, may be major inducers of uPAR release from SCC and melanoma cells, thus modulating tumor invasion and metastasis.

P242

Ser102 phosphorylation of the transcription factor YB-1 enhances proliferation and invasive growth of melanoma cells

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Y-box binding protein 1 (YB-1) is an oncogenic transcription and translation factor and is overexpressed in several types of cancer. Our previous data indicated that YB-1 is upregulated and translocated to the nucleus during melanoma progression and that YB-1 is an important transcription factor regulating proliferation, survival, migration, invasion and chemosensitivity of melanoma cells. Furthermore, we showed that during melanoma progression in patient samples total YB-1 expression as well as phosphorylation at Ser102 in the YB-1 protein is increased. Nuclear translocation and transcriptional activation of YB-1 was reported to be mediated by Ser102 phosphorylation in the nucleic acid binding domain. We hypothesized that primarily the activation of YB-1 by phosphorylation and not total YB-1 abundance augments the functional effects of YB-1 as a transcription factor during melanoma progression towards metastasis. Indeed, the specific inhibition of Ser102 phosphorylation of YB-1 by a phospho-mimetic YB-1 decoy peptide blocks YB-1 transcriptional activity and significantly reduces melanoma cell proliferation as well as melanoma cell migration and invasion. Melanoma cells with stable overexpression of either a dominant-negative or a dominant-active mutant of YB-1 (Ser102Ala and Ser102Asp, respectively) were created in order to reassess the relevance of these findings and to evaluate their effects on the transcriptional and translational activity of YB-1.

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Melanoma treatment with DBD and APPJ in a mouse model

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Objectives: To compare cold plasma therapy using two different sources with electrotherapy and chemotherapy in a mouse model

Materials and Methods: B16-F10 melanoma tumours cells (105 cells in 100 l) were subcutaneously injected into the hind leg of C57BL/6N mice. The mice received 6 min of DBD and 5 min with APPJ-treatment. Plasma treatment was daily repeated over 5 days. Tumour volumes were calculated using the formula $V = (a*b2)/2$.

Chemotherapy (single shot) was realized with i.v. bleomycine (6 mg/kg body weight) and electrotherapy (single shot) using Cliniporator® (IGEA, Carpi, Italy), delivering defined pulses creating significant electrical fields via parallel plate electrodes (16 pulses, pulse length 100 s, 1000 V/cm).

Results: Both plasma sources as single treatment and also in combination with electrotherapy showed a significant delay of tumor volume progression compared to untreated controls. Additional, the survival was significantly improved, when cold plasma treatment was combined with i.v. bleomycine and electrotherapy. Neither electrotherapy nor chemotherapy alone significantly changed the outcome of melanoma in the mouse model (tumor progression and survival).

Conclusion: In the mouse model, two completely different plasma sources proved high effectiveness of cold plasma against melanoma and support the previously reported data of Vandamme et al. [1,2]. In addition, the plasma effectiveness can be further improved in combination with electro- and chemotherapy, showing synergistic antimelanoma efficacy. In future, cold plasma may play a significant role in curative and palliative tumor management. As an alternative to conventional treatments, cold plasma therapy may be applied i.e. when contraindications do not allow general anesthesia, or in case of multiple, bucky and otherways untreatable tumors and metastases. Cold plasma acts synergistically to bioelectric and chemo-therapy, seemingly – besides antinuclear lethal effects – adding vascular effects of both treatments (i.e. vasoconstriction, endothel toxicity, microthrombosis, cytoskeletal disarrangement, hemostasis).

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Regulatory mechanisms involved in activation of PI3K/AKT-signalingin melanoma cells

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The PI3K/AKT signaling pathway is involved in melanoma survival, proliferation, and therapy resistance and is considered to be constitutively activated in melanoma cells. However, several studies indicated that the PI3K/AKT signalling pathway is not constitutively activated in metastatic melanoma cells in patients and that there is a strong correlation of PI3K/AKT activation and the localisation of metastases in melanoma patients. This suggests that micro-environmental factors in the respective tissues influence activation of PI3K/AKT signaling in melanoma cells. Recent data indicate that amplified PI3K/AKT signaling via an upregulated expression of the IGF1R on melanoma cells is involved which even confers resistance to MAPK-inhibition. Since known ligands for IGF1R are not expressed by melanoma cells itself, amplified PI3K-signaling via the IGF1R might either be achieved by a paracrine mechanism in the tissue microenvironment, i.e. dermal fibroblasts secreting IGF-1, or an intrinsic intracellular regulatory mechanism of melanoma cells. In this project we analysed the regulatory mechanisms involved in activation of PI3K-signaling in melanoma cells and the impact on invasion, metastasis and therapy resistance of melanoma cells. We focussed on the expression analysis of key components of the IGF1R/PI3K/AKT pathway in different human melanoma cell lines and analyzed how the expression level changes after stimulation with IGF1 and insulin. We also investigated the role of IRS1 and IRS2 as mediators of IGF1R signaling using IRS1/2 specific siRNAs. Moreover, we checked the effect of the microenviromental factors on the activation of PI3K/AKT signaling in melanoma cells. Our results indicate that activation of PI3K/AKT signaling is highly regulated *in vivo* and *in vitro* in melanoma cells. Although both – IGF1 and insulin – could activate PI3K/AKT pathway through their own receptors, the adaptor proteins IRS1 and IRS2 seems to be differentially regulated in melanoma cells and both proteins have different functional effects on activation of PI3K/AKT signaling. Moreover, co-culture of human melanoma cells with secreted factors of dermal fibroblasts increased resistance towards the BRAF-inhibitor vemurafenib. These data indicate that key components of the IGF1R-signaling pathway are differentially involved in activation of the PI3K/AKT signalling pathway in melanoma.

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Melanoms resist adoptive T-cell therapy through inflammation-induced reversible dedifferentiation

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Melanoma immunotherapy with adoptively transferred T-cells targeting melanocytic antigens can achieve remissions in patients with metastatic disease, but tumors frequently relapse. Hypotheses explaining acquired therapy resistance include the selection of antigen-deficient tumor cell variants and the suppression of T-cell effector functions in the tumor microenvironment. To gain further insights in the mechanisms underlying tumor relapse, we established an effective adoptive T-cell immunotherapy protocol in the genetically engineered Hgf-Cdk4 mouse melanoma model that faithfully recapitulates tumor regression, remission and relapse as seen in patients. Hgf-Cdk4 mice bearing palpable primary melanomas in the skin received an adoptive transfer of nave gp100-specific pmel-1 TCRtg T-cells that were activated *in vivo* with Ad-gp100 followed by adjuvant administration of CpG and pI:C. This caused melanoma regression and remission in the majority of mice. Histopathologic analyses of melanomas that relapsed after several months of remission revealed focal absence of gp100 expression in amelanotic tumor areas associated with increased immune cell infiltration. Experiments with the transplantable Hgf-Cdk4 melanoma cell line HCmel3 recapitulated tumor regression, remission and relapse and confirmed the development of acquired resistance to gp100-specific T-cell therapy associated with partial loss of target antigen expression in an inflammatory microenvironment. HCmel3 cells isolated from relapsed tumors showed a dedifferentiated phenotype with a strongly reduced ability to stimulate pmel-1 T-cells *in vitro*. In serial transplantation experiments we found that HCmel3 cells switched between a differentiated and a dedifferentiated phenotype in response to T-cell driven inflammatory stimuli. Whole genome mRNA expression analyses of control, relapsed and re-transplanted HCmel3 melanomas revealed a reversible down-regulation of 25 pigmentations genes in relapsed tumors accompanied by a broad up-regulation of immune response genes. To subtract immune cell associated profiles we integrated gene expression data from *in vitro* cultures of dedifferentiated HCmel3 relapse lines and identified a set of cell adhesion, migration and extracellular matrix genes that is reversibly up-regulated in relapsed HCmel3 melanomas *in vivo*. These results indicate a mesenchymal-like phenotype switch of melanoma cells in the context of an inflammatory microenvironment. Taken together, these observations suggest that immunoselection of tumor cell variants with persistent genetic loss of the antigen, as predicted by the immunoediting theory, is not responsible for acquired resistance to gp100-specific T-cell immunotherapy. Instead, the experimental results indicate that tumor relapse after initially successful T-cell immunotherapy involves a reversible shift of melanoma cells towards a dedifferentiated state in response to T-cell driven inflammation in the microenvironment.

P246 (O33)

Generation of multifunctional immunostimulatory siRNAs for melanoma targeting uPAR

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Nucleic acids represent an important pathogen-associated molecular pattern by which the host senses a viral infection. Short double-stranded RNA molecules can be recognized by the cytosolic receptor RIG-I when carrying a triphosphate moiety at their 5′ end. RIG-I is ubiquitously expressed and its activation elicits an antiviral response in several cell types, including tumor cells. The antiviral response consists of induction of interferons and proinflammatory cytokines and of activation of proapoptotic molecules. Activation of innate immunity and apoptosis are also relevant for tumor therapy. Furthermore, double-stranded short triphosphate RNAs can be designed to trigger RNA interference. Silencing of a therapeutic relevant gene adds another function to the compound, which leads to a multifunctional RNA that is optimized for a certain tumor type.

Based on our previous work, we generated multifunctional RNAs for melanoma therapy by synthesizing triphosphate-conjugated siRNAs targeting the urokinase-like plasminogen receptor (uPAR). Due to its pro-metastatic role and its importance for melanoma survival, uPAR is an attractive therapeutic target. Inhibition of uPAR induces apoptosis via activation of the tumor suppressor p53, which should synergize with apoptosis upon RIG-I activation, which is p53-independent. Two uPAR-targeting immunostimulatory siRNAs were generated by *in vitro* transcription. Both efficiently reduced uPAR expression in melanoma cells and induced expression of interferon-beta. Triphosphate-conjugated uPAR siRNAs strongly induced apoptosis in melanoma cells and they were more effective compared to non-immunostimulatory uPAR siRNAs or non-targeting immunostimulatory RNAs. Together, the data suggest that immunostimulatory siRNAs targeting uPAR represent an attractive tool to activate multiple pathways that synergize for therapeutic purposes.

P247

Epigenetic regulation of Brn3a in melanocytic cells

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During development, melanocytes and neural cells develop from common progenitor cells located in the neural crest. The transcription factor Brn3a is expressed in neural crest cells and is important for survival of neuronal cells. We observed that Brn3a is highly expressed in human melanoma cells, but not in primary human melanocytes. Knock-down experiments revealed that Brn3a is essential for melanoma cell proliferation and survival. The factors that cause Brn3a expression in melanoma are not known. Here, we addressed epigenetic regulatory mechanisms in Brn3a-negative melanocytes and other cells of the skin.

Long-term gene silencing is often maintained by DNA methylation. The Brn3a gene locus contains two large CpG islands within the 5′ region. We tested whether Brn3a is silenced in primary human melanocytes via DNA methylation. Treatment with DNA-demethylating reagents caused a 3–6-fold increase in Brn3a expression in melanocytes whereas fibroblast showed less upregulation. However, Brn3a upregulation in melanocytes did not reach the levels of melanoma cells.

Next, melanocytes were treated with the histone deacetylase (HDAC) inhibitor Trichstatin A. Interestingly, Trichostatin A highly upregulated Brn3a and expression levels reached the one of melanoma cells. In contrast, other developmental factors like Brn2 and Sox9 were not upregulated by Trichstatin A. Similar results were observed with another inhibitor specific for HDAC1. Treatment of melanoma cells only slightly increased Brn3a levels. In primary fibroblasts, low upregulation was observed on the mRNA level, which did not lead to detectable protein amounts.

Together, the data suggest that – besides DNA methylation – HDAC1 plays an important role in the regulation of Brn3a in melanocytic cells. In addition, Brn3a expression is controlled by lineage specific factors, because HDAC inhibition in non-melanocytic cells like fibroblasts did not lead to substantial Brn3a levels. Therefore, besides genetic mechanisms, epigenetic mechanisms play a critical role in Brn3a regulation.

P248 (O08)

TNF-α reversibly shifts melanoma cells towards a dedifferentiated state leading to selectively impaired recognition by CD8+ T-cells specific for pigmentation antigens

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Treatment of mice bearing established Hgf-Cdk4 melanomas with an adoptive cell therapy protocol targeting the pigment cell antigen gp100 causes tumor regression and remission. However, some mice acquire resistance to T-cell therapy leading to melanoma relapse associated with loss of gp100 expression in an inflammatory tumor microenvironment. Using the transplantable Hgf-Cdk4 melanoma cell line HCmel3 we observed that loss of target antigen expression and sensitivity to T cell therapy were largely restored upon transplantation. These findings led us to hypothesize that the melanocytic differentiation status of HCmel3 cells may be dynamically regulated in the tumour microenvironment by immune cell-derived proinflammatory mediators. Histopathological investigations of relapsed HCmel3 melanomas revealed increased expression of Ngfr, a marker for neural crest progenitors, on gp100low cells preferentially in areas with intense immune cell infiltration. Consistent with our hypothesis we observed that HCmel3 cells up-regulated Ngfr and down-regulated gp100 expression following exposure to culture supernatants conditioned by activated T cells or macrophages *in vitro*. We could identify Tnf-α secreted by these immune cells as an important proinflammatory mediator that induced reversible dedifferentiation of HCmel3 cells. As a consequence Tnf-α treated HCmel3 cells were poorly recognized by gp100-specific TCR-transgenic pmel-1 T cells. Gene expression profiling demonstrated that the transcriptional response to Tnf-α recapitulated the downregulation of 25 pigmentation genes and the mesenchymal-like phenotypic switch observed in HCmel3 cells from relapsed melanomas compared with parental HCmel3 cells. Similar to our observations in mouse cells we found that TNF-α also transiently converted differentiated gp100+ Ngfrlow human MZ7-MEL melanoma cells into undifferentiated gp100low Ngfr+ cells. This led to selectively impaired recognition of MZ7-MEL by autologous T cells specific for melanocytic antigens while recognition by T cells specific for individually mutated non-melanocytic antigens was not affected. In conclusion we show the proinflammatory cytokine TNF-α secreted by macrophages and T-cells in the tumor microenvironment reversibly shifts mouse and human melanoma cells towards a dedifferentiated state leading to impaired recognition by melanocyte-specific T cells. Our data support a model where melanoma cells exist in a dynamic, interconvertible equilibrium between differentiated and dedifferentiated subpopulations that rapidly adapts to inflammatory signals in the environment. Similar dynamic changes in the tumor microenvironment as a mechanism of therapy resistance are currently also emerging for targeted inhibitors of signal transduction pathways in BRAFV600E mutant melanomas.

P249 (O19)

The neural transcription factor SOX10 promotes melanoma cell invasion and regulates migration-associated genes

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The neural transcription factors SOX9 and SOX10 are crucial for the embryonic development of melanocytes and have been shown to be expressed in melanoma. Both SOX9 and SOX10 can activate microphthalmia-associated transcription factor and melanogenic enzymes. However, the role of these factors and their further target genes in melanoma are not entirely clear.

We have analyzed SOX9 and SOX10 mRNA and protein expression in various melanoma cell lines and found an inverse expression of both factors in the majority of these lines. Melanocytes displayed low SOX9 and high SOX10 levels compared to most melanoma cells.

We investigated the phenotype of melanoma cells upon SOX10 inhibition and observed a significant reduction of cell viability and an increase in apoptosis leading to about 60% dead cells 5 days after siRNA transfection. Furthermore, SOX10 inhibition decreased melanoma cell migration as determined by wound healing and transwell invasion assays. Spheroid assays demonstrated that SOX10 inhibition prevented the proper formation of spheroids in a 3D model of melanoma independent of cell death. Moreover, SOX10 was required for tumor growth and invasiveness *in vivo*, as determined in the rhombencephalon of the chick embryo.

To clarify the mechanism how SOX10 affects melanoma migration, we analyzed putative SOX10 target genes in melanoma. The receptor tyrosine kinase ERBB3 has been shown to be regulated by SOX10 in melanocytes and has been associated with metastatic progression in melanoma. Indeed, ERBB3 levels correlated with SOX10 levels in the tested melanoma cell lines and inhibition of SOX10 by RNA interference resulted in reduced expression of ERBB3.

In addition, we identified melanoma inhibitory activity (MIA), a key molecule involved in progression and metastasis of melanoma, as a new target gene of SOX10. Strikingly, SOX10 and MIA expression levels correlated in all examined melanoma cell lines. Inhibition of SOX10 strongly reduced MIA expression and activity of MIA promoter constructs, indicating a direct regulation of MIA by SOX10.

In summary, we have identified migration-associated genes like MIA and ERBB3 as new target genes of SOX10 in melanoma and demonstrate that SOX10 is required for melanoma cell invasion.

P250

Knockdown and pharmacological inhibition of LRAT lead to increased all-trans retinoic acid levels and restore retinoid sensitivity in malignant melanoma cells

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Vitamin A (all-trans retinol, ATRol) serves as a substrate for several derivates (retinoids), which are able to influence cell growth, differentiation and apoptosis by controlling various signaling pathways. Retinoids like all-trans retinoic acid (ATRA) are successfully used for pharmacological treatment of several forms of cancer but human malignant melanoma is insensitive towards retinoid therapy. Our aim was to investigate the significance of vitamin A metabolism for tumor biology and retinoid resistance in human malignant melanoma.

Recently, we identified human melanoma specific alterations in the expression and activity of enzymes involved in retinoid metabolism. One of these key enzymes is lecithin/retinol acyltransferase (LRAT), which is expressed in melanoma cells but not in melanocytes catalysing the esterification of ATRol. In this study we could show that stable LRAT knockdown (LRAT KD) in the human melanoma cell line SkMel23 leads to low biological inactive retinyl ester. A high ATRA concentration could be found by pharmacological inhibition of LRAT activity by all-trans retinyl alpha-bromoacetate in melanoma cells. LRAT KD restored their sensitivity to retinoids analysed by cytotoxicity tests and proliferation assays and led to changes in gene regulation after retinoid treatment analysed by microarray analysis. These results were confirmed by qRT-PCR revealing an upregulation of retinoid-regulated genes like CYP26A1 and STRA6 expression in LRAT KD cells. This ATRA-induced gene regulation mediated the depletion of added ATRol in LRAT KD cells. Therefore, STRA6 and CYP26A1 are further candidate genes that could mediate retinoid resistance in malignant melanoma.

We propose that the removal of ATRol in melanoma cells by LRAT leads to a disturbance in cellular retinoid level and protects tumor against retinoids. Consequently, the decreasing cellular amount of ATRA and its precursor molecules should result in a change of gene regulation. Inhibition of LRAT activity could be a promising strategy to overcome retinoid insensitivity in human melanoma cells.

P251

Vemurafenib induces senescence features in melanoma cells

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A large proportion of human melanomas harbor a mutation leading to permanent activation of the serine/threonine kinase BRAF, and as a consequence, they have developed dependence on BRAF/MAPK signaling. Accordingly, BRAF inhibitors such as Vemurafenib show a good anti-tumorigenic effect on metastases with the BRAFV600E mutation. Although an initial periode of sustained tumor regression is usually observed after Vemurafenib treatment, tumors often relapse at the same site, and apoptosis induction of melanoma cells *in vitro* is incomplete. Here, we demonstrate, using a large panel of melanoma cell lines, that Vemurafenib induces features of stress-induced senescence in addition to apoptosis. This senescence phenotype is characterized by heterochromatin formation, changes in cell shape and increased senescence-associated β-galactosidase activity. Importantly, senescence induction by BRAFV600E inhibition was also detected in human melanoma cells xenografted into nude mice. Our observations provide a possible explanation for the lack of complete and durable pro-apoptotic effect of Vemurafenib in patients.

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Senescence and apoptosis response to genotoxic stress in melanoma cells

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The human genome is constantly exposed to genotoxic stresses such as ultraviolet light, reactive oxygen species (ROS), chemotherapeutic agents as well as biological mutagens. To ensure the integrity of the genome cells have evolved a sophisticated safety system that can implement a cell cycle arrest to allow the repair of the incurred damage. In the case of irreparable damage, the cell will be permanently retracted from the pool of dividing cells by the induction apoptosis or senescence. In the present study we used the chemotherapeutic agents taxol and cisplatin to trigger a senescence-like growth arrest or apoptosis in a panel of melanoma cell lines and studied the underlying signaling events. Using western blot and immunofluorescence analysis we demonstrate that the DNA damage response and subsequent activation status of the p53-p21 pathway determines the cell fate by regulating the balance between cellular senescence and apoptosis. Interestingly, the activation status of ATM (ataxia-telangiectasia mutated) and ATR (ATM and Rad3-related) protein kinases, which in turn activate checkpoint-1/2 (CHK1/2) kinases leading to phosphorylation and thereby stabilization of p53 and subsequently p21 is depending on the type and level of genotoxic stress and differs between different melanoma cell lines. Suppression of ATM and ATR kinase activity using the inhibitor caffeine leads to dose dependent reduction of apoptosis in favor of senescence. Our results suggest that the DDR pathway can determine, the choice between senescence and apoptosis in response to genotoxic stress in melanoma cells.

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The BRAFV600E kinase inhibitor Vemurafenib potently induces endoplasmic reticulum (ER) stress and ER stress-mediated apoptosis

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Although the BRAFV600E kinase inhibitor, vemurafenib, has remarkable antitumor activity in patients with BRAFV600E-mutated melanoma its effects are limited by the onset of drug resistance. The aims of the present study were to determine the mechanisms of action and resistance to vemurafenib.

The BRAFV600E kinase inhibitor vemurafenib inhibited growth, induced caspase-dependent apoptosis and upregulated the ER stress-related genes p8, CHOP, ATF4, ATF3 and TRB3 mRNA levels exclusively in BRAFV600E mutated melanoma cell lines. Apoptosis was correlated with the increased abundance of the proapoptotic BH3-only proteins Bim-particularly Bim short, PUMA and Noxa. Knockdown of Bim significantly reduced vemurafenib-induced apoptosis.
Vemurafenib raised cytosolic Ca2+, suppressed the endoplasmic reticulum (ER) chaperone protein GRP78, induced phosphorylation of the translation initiation factor eIF2α and induced expression of the transcription factor XBP1 spliced. Knockdown of the ER stress-related protein, ATF4, significantly reduced vemurafenib-induced apoptosis. Thus, vemurafenib potently induced ER stress, which triggered apoptosis in BRAFV600E melanoma cells. In melanoma cells with low sensitivity or resistance to vemurafenib, the classical ER stress inducer, thapsigargin, augmented or induced apoptosis. Thapsigargin overcame vemurafenib resistance, and may be useful in combination therapies.

P254

A critical role for Stat1 in the control of metastases

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Immunotherapy with tumor-specific Th1 cells reduces tumor burden in humans with melanoma and in mice with neuroendocrine cancers. In mice, neuroendocrine cancers, develop because of expression of oncogenic large T antigen 2 (Tag) of the Simian virus 40 under the rat insulin promoter 1 (RIP1-Tag2 mice). Previous studies indicated that cancer control by Th1 cells strictly depends on two cytokines, interferon-gamma (IFN-γ) and tumor necrosis factor (TNF). To examine the mechanisms underlying the cancer control by Th1 cells and the impact of the microenvironment during tumor progression, we generated tumor bearing mice lacking the signal transducer and activator of transcription 1 (Stat1), a critical component of the IFN-signaling cascade. While Th1 cells were capable of doubling the total life span of Stat1-competent RIP1-Tag2 mice, Th1 cells failed to prolong the survival of RIP1-Tag2xStat1.ko mice, suggesting a critical role for Th1 cell-derived IFN-γ-signaling in therapeutic cancer control. More detailed analysis revealed that RIP1-Tag2xStat1.ko mice developed the same number of islet carcinomas, similar changes of the tumor-blood marker and had a similar survival as untreated RIP1-Tag2 control mice. In contrast to normal cancer developing RIP1-Tag2 mice, where Th1 cells prevented cancer development, Th1 cell did not affect the course of cancer development in RIP1-Tag2xStat1.ko mice. Most important however was the impact of Stat1 on the development of metastases. While, RIP1-Tag2 mice never develop detectable metastases (<1%), 50% of RIP1-Tag2xStat1.ko mice developed strictly lymphogen metastasis in mesenteric lymph nodes. To uncover the mechanisms promoting the metastatic potential of the primary tumors, we investigated the gene expression pattern of the neuroendocrine cancers, focusing on epithelial-mesenchymal transition. The expression data strongly suggest that Stat1 critically controls cell motility and invasiveness through its impact on epithelial differentiation markers like E-cadherin, Keratin-7 and Desmoplakin on one side and on mesenchymal differentiation marker Foxc2 on the other side. Others had recently shown that IFN-γ-signaling strongly affects 'cancer immune-editing', cancers from RIP1-Tag2xStat1.ko mice express a more divers pattern of tumor-associated antigens then RIP1-Tag2 cancers from wild-type mice. To directly address this question, and to test whether tumor-immunity can overcome this more aggressive phenotype of Tag2xStat1.ko cancers, we injected either RIP1-Tag2 cancers or RIP1-Tag2xStat1.ko cancers into immunocompetent WT mice. Five out of eight RIP1-Tag2 cancers grew at ectopic sites in immunocompetent mice, whereas 0/6 transferred RIP1-Tag2xStat1.ko grew in syngeneic immunocompetent mice. Inhibition of Stat1-signaling promoted epithelial-mesenchymal transition. Moreover, Stat1-signaling impaired the cancer immune-editing. Thus, the immune system can overcome the disadvantage resulting from deficient Stat1-signaling and the enhanced epithelial mesenchymal transition by recognizing the extended antigen-pattern of Stat1-deficient cancers and reject the developing cancers even more efficiently, in the context of an appropriate adaptive immunity.

P255

Reversible epigenetic silencing of NKG2D ligands MICA and MICB in Merkel cell carcinoma

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Merkel cell carcinoma (MCC) is a highly aggressive neuroendocrine skin cancer. In about 85% of MCC tumors the mercel cell polyomavirus (MCPyV) can be detected. Despite this virus infection and the subsequent expression of immunogenic viral proteins, MCCs are able to evade the immune system. One of the early responders to virally infected cells and tumor formation are natural killer cells (NK cells). Infected or stressed cells express activating NKG2D receptor ligands like major histocompatibility complex class I-related chain A and B (MICA/B), triggering an activation of NK cells and cytotoxic lysis of the stressed cell. In the present study we therefore examined the expression and regulation of MICA/B molecules on MCCs.
We observed that all eight studied MCPyV positive MCC cell lines expressed none or very little MICA and MICB on the mRNA and protein level. Since MICA/B expression can be silenced epigenetically by histone deacetylation of the promoter region, we analyzed the effect of HDAC inhibitors like SAHA (Vorinostat) on MCC cell lines. To this end, SAHA treatment resulted in an increased overall histone acetylation level in all MCC cell lines which was associated with detectable MICA/B expression. Accordingly, ChIP analysis demonstrated enhanced Histone H3 acetylation of the MICA/B promoters after SAHA treatment. Because SP1 was previously described as an activating transcription factor for the MICA/B promoter we subsequently tested whether the observed effect could be reversed by treatment with the SP1 specific inhibitor mithramycin A (MA). Surprisingly, the combination of SAHA and MA revealed a synergistic effect on the MICA/B expression in all eight cell lines. Western Blot analysis revealed that each substance alone could decrease SP1, whereas it was completely diminished by the combination. This data imply that SP1 – unlike in other types of cancer – is acting as a transcriptional repressor for the MICA/B promoter in MCCs.
Notably, all three MCPyV negative cell lines expressed MICA/B per se, but very little SP1 indicating that SP1 and subsequently MICA/B expression might be influenced by the presence of MCPyV. Indeed, an inducible knock down of the virus protein large T Antigen resulted in lower SP1 levels in three out of four examined MCC cell lines, which however, was not sufficient for a re-expression of MICA/B in those cells. Importantly, SAHA and MA induced MICA/B expression on two MCC cell lines rendered them more prone to NK cell mediated tumor cell lysis than untreated cells.
Our results indicate that MCPyV positive MCC cell lines evade the immune system by silencing the NKG2D Ligand expression. Thus, inducing MICA/B expression in their tumors by treatment with HDAC inhibitors in combination with mithramycin A might be a promising therapy for MCC patients.

P256 (O05)

Th1 cells induce TNFR1-dependent tumor dormancy

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Genome-wide analysis of malignant tumours revealed multiple alterations in cell cycle control genes in all types of cancers. These mutations still remain susceptible to therapies, and small molecules targeting cell cycle control, such as BRAF inhibitors, improve the clinical outcome of cancer patients. Surprisingly, many cancer immunotherapies do not cause cytotoxic cancer elimination but arrest cancer growth or induce slow cancer regression. Moreover, where studied, growth arrest and cancer regression correlate with tumor-specific, interferon (IFN)-γ- and tumor necrosis factor (TNF)-producing CD4-positive (Th1) cells rather than cytotoxic T cells.
Here, we analyzed the effects of cancer-specific Tag-Th1 cells on tumour cell proliferation and tumour size in multistage carcinogenesis using RIP1-Tag2 mice expressing the oncoprotein T antigen (Tag) in their Langerhans islets. Interestingly, Tag-Th1 cells doubled the survival of RIP1-Tag2 mice. Treatment also enabled RIP1-Tag2 mice to control blood glucose levels, and reduced the tumour burden of the mice, both effects being dependent on TNF receptor 1 (TNFR1). To investigate the underlying mechanisms, we analyzed cancers from RIP1-Tag2 or TNFR1−/−xRIP1-Tag2 mice after 6 weeks of Tag-Th1 treatment. Immunofluorescence staining revealed that the proliferation marker Ki67 decreased from 30% in sham-treated mice to 3% in Tag-Th1-treated mice, whereas the cell cycle inhibitor p16Ink4a was significantly increased by the treatment. In accordance with the lack of therapeutic effect, transfer of Tag-Th1 cells into TNFR1−/−xRIP1-Tag2 mice did neither upregulate p16Ink4a nor significantly reduce Ki67 staining. To further substantiate these findings, we isolated β-cancer cells from sham- or Tag-Th1 cell-treated mice. β-cancer cells from sham-treated RIP1-Tag2 mice first suffered a critical loss and then re-initiated proliferation whereas β-cancer cells from RIP1-Tag2 mice treated with Tag-Th1 cells failed to proliferate over three passages. In clear contrast, TNFR1-negative β-cancer cells grew exponentially *in vitro* even when derived from Tag-Th1 cell-treated mice.
Taken together, our data show that Th1-immunity restrains cancer proliferation through TNFR1-dependent, p16Ink4a-mediated cell cycle arrest. This provides a long-searched direct mechanism explaining the anti-proliferative effects of Th1-immunity on growing cancers.

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A function for Rac1 in the regulation of UV induced keratinocyte apoptosis

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The small GTPase Rac1 belongs to the family of Rho GTPases and has important functions in actin cytoskeletal rearrangement as well as cell adhesion and migration. It has also been shown to play an important role in the establishment of the epidermal barrier by regulating keratinocyte adhesion and spreading and the maintenance of the epidermal stem cell pool. We have recently shown an additional function for Rac1 in hair differentiation. Rac1 overexpression has been linked to squamous cell carcinomas of the epidermis and mucosa in man. In experimental models of chemically induced skin carcinogenesis in mice, Rac1 is required for the development of papillomas and carcinomas. Since the most relevant carcinogen to human skin is UV light, we set out to investigate the role of Rac1 in UV induced skin carcinogenesis. For that we generated mice with epidermis specific deficiency of Rac1 as well as transgenic mice expressing activating and inhibitory mutants of Rac1 in the epidermis. These mice were crossed to mice expressing the complete early region of human papilloma virus 8 (HPV8) under the control of the keratin 14 promoter. When these mice were irradiated with a single dose of UVA/ UVB light, all of them developed skin papillomas. In contrast, only 8% of the mice expressing the inhibitory mutant N17Rac1 in addition to HPV8, showed skin papilloma development. We have asked by which mechanism inhibition of Rac1 suppressed tumour formation in these mice. *In vitro* and *in vivo* studies with N17Rac1 expressing epidermal keratinocytes and in mice with epidermis specific deficiency of Rac1 revealed that the presence and activity of Rac1 in epidermal keratinocytes protects these cells from UV induced apoptosis. This function of Rac1 is new and specific for UV light induced apoptosis, since Rac1 did not protect from TNF- α induced dell death. We are currently investigating into the molecular mechanisms that link the role of Rac1 in the regulation of apoptosis to skin tumour formation.

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An Angiopoietin-2 high Tie2 low endothelial phenotype correlates with an aggressive clinical course in primary cutaneous B-cell lymphomas [Abstract of the Working group of cutaneous lymphomas of the Arbeitsgemeinschaft für Dermatologische Forschung (ADF), Germany]

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Angiopoietin-2 (Ang-2), the ligand of Tie2 receptor, represents a pivotal molecule during angiogenesis. However Ang-2 signaling is dichotomous with a destabilizing effect in Tie2 high stalk/phalanx endothelial cells (EC) and a sprouting effect in Tie2 low tip cells during physiological angiogenesis. Ang-2 induced sprouting in Tie2 low tip cells requires expression of the angiogenic integrins αvβ3/αvβ5/α5β1 and is accompanied by FAK phosphorylation at Tyrosine397 (p-FAK[Tyr397]). Tie2 low EC have been described in several malignancies but the relevance of Ang-2 stimulation of Tie2 low EC in tumors remains an enigmatic question in the understanding of tumor angiogenesis. The aim of this study was to investigate if tumor malignancy correlates with Ang-2 signaling in Tie2 low EC. Primary cutaneous B-cell lymphoma (PCBCL) of indolent behavior were compared with aggressive PCBCL for their vascular network morphology, Tie2, Ang-2, angiogenic integrin and p-FAK[Tyr397] expression. Aggressive PCBCL showed significant smaller, more often non-lumenized vessels defining tip cell morphology. Abundant Ang-2, significant more Tie2 low EC and significant higher EC expression of the angiogenic integrins were observed in aggressive PCBCL. Enriched p-FAK[Tyr397] in sprouting vessels was only detectable in aggressive PCBCL. Concordantly, significant more sprouting vessels were found in aggressive PCBCL. In summary, an aggressive clinical course correlates with an Ang-2 induced Tie2 low EC phenotype in PCBCL.

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Melanoblast related cells (MBrc) express Neuritin (NRN1) commensurate to melanoma cell lines

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Cells in embryogenesis harbor several features like high proliferative capability, which are also found in cancer cells. In embryogenesis these characteristics are strictly controlled whereas in cancer this regulation is lost. In this project we focus on a detailed understanding of differentiation and migration of melanocytes in embryogenesis compared to melanoma cells to determine molecular changes in regulation.
Here, under the influence of medium supplemented with Stem Cell factor, Endothelin-3 and Fibroblast Growth Factor-2 melanoblast in culture were generated out of melanocytes. By cDNA Array

analysis we compared the gene profile of melanocytes, corresponding melanoblast related cells (MBrc) and several melanoma cell lines.
Interestingly, one gene NRN1 (CPG15; candidate plasticity-related gene 15) of the neutrophin family was up-regulated in MBrc and melanoma, respectively compared to melanocytes. NRN1 is a glutamate and neurotrophin receptor (p75NTR and Trk) target gene encoding a neuronal protein that functions extracellular to modulate neurite outgrowth. Beside this chemo-attractive function NRN1 regulates proliferation and apoptotic processes of neurons. Further studies suggest that NRN1 might be involved in angiogenesis. All these diverse roles of NRN1 could also be important for melanoma. Therefore, NRN1 is as newly detected secreted factor of MBrc and melanoma cells whose function must be clarified for this kind of skin cancer. Our results showed the involvement of NRN1 in migration and anchorage independent growth of melanoma.

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Clinical scale generation of Survivin-specific CD4+, interferon-γ-producing T helper lymphocytes for adoptive T-cell transfer to treat metastatic malignant melanoma

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Adoptive T-cell transfer is considered as a promising therapeutic option for the treatment of patients with metastatic malignant melanoma. T lymphocytes for adoptive T-cell transfer are generated *ex vivo* and re-infused into the patient. Recent evidence suggests that CD4+ T helper (Th1) lymphocytes are of great importance for the treatment of metastatic melanoma as they orchestrate the response of cytotoxic T lymphocytes and can impair tumor growth and angiogenesis through cytokine signaling. We designed a GMP conform protocol to generate Survivin-specific Th1 lymphocytes. Survivin is considered as an important therapeutic target in the treatment of human malignancies as the expression is very low in most normal tissues. In sharp contrast, Survivin is strongly expressed in most tumor entities. Furthermore, Survivin is involved in signaling pathways that promote tumorigenesis. We primed PBMCs from healthy donors with Survivin 15-mere overlapping peptide mixes. After 2 weeks of stimulation with IL-2 und IL-7, we enriched IFN-γ producing cells with the IFN-γ capture technique. Cells were expanded with a defined cytokine cocktail over 2 weeks prior to analysis. We could generate a cell product with up to 3×10^9 cells. Phenotypical and functional analysis showed that the predominant population were Survivin-specific Th1 lymphocytes producing IFN-γ, IL-2 and TNF-α. Tolerance inducing cytokines like IL-4 or IL-10 were absent. Enrichment of regulatory T lymphocytes was excluded by FACS-staining. Furthermore we found that the generated cells produced a Th1 cytokine profile after peptide-specific restimulation. Moreover Th1 cell-derived cytokines such as Interferon-γ impaired tumor growth and arrested the cell cycle of melanoma cells.
In summary, GMP conform generation of Survivin-specific Th1 lymphocytes for the use in immunotherapy against metastasized malignant melanoma was established and the effects of Th1 cytokines on melanoma cells were analysed.

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The soluble form of RAGE (Receptor for advanced glycation end-products) as a prognostic marker in melanoma

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Melanoma initiation, growth and progression have been related to microenvironmental factors orchestrating tumor-stroma interaction. However, the importance of these factors for predicting disease progression in a clinical setting have not yet been established.
We have recently demonstrated that the receptor for advanced glycation end-products (RAGE) is a driver of inflammation-associated tumorigenesis by sustaining a chronic inflammatory tumor-microenvironment.
This study was aimed to identify a new prognostic biomarker of melanoma by a hypothesis-driven approach linking the RAGE pathway with clinical outcome of melanoma patients. Moreover, the new candidate, the soluble form of RAGE (sRAGE), was validated compared with established markers.
Plasma concentrations of the candidate marker protein sRAGE and known biomarkers S100B, and lactate dehydrogenase (LDH) as well as C-reactive protein (CRP) were measured in 279 serum samples from 217 melanoma patients (*in situ* melanoma = 3; stage I = 49; stage II = 36; stage III = 55; stage IV = 74) using immunoassays (ELISA). sRAGE-specific ELISA measurements revealed significantly decreased plasma levels of sRAGE in melanoma patients compared to healthy controls ($P < 0.00001$) as well as in melanoma patients with progressive disease within 90 days after blood draw compared to patients without progression ($P = 0.028$). Moreover sRAGE levels lower than 400pg/mL were found to be an independent marker for overall survival (OS) and progression-free survival (PFS) in stage I-IV ($P = 0.0017$; $P = 0.0159$) and in stage IV ($P = 0.005$; $P = 0.002$) patients.
In conclusion, we provide clinical evidence for a novel role of RAGE signaling in driving melanoma growth and development by highlighting the importance of melanoma-stroma interaction in a RAGE-dependent manner. Moreover, we shed light on RAGE signaling as a novel strong prognostic marker in melanoma as well as a promising target for anti-melanoma therapy.

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TH1 cell cytokines stabilize the p16Ink4a/Rb pathway in β-cancer cells

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p16Ink4a/retinoblastoma protein (Rb)-dependent cell cycle regulation is frequently impaired in various tumors, including HPV-positive epithelial cancers. In RIP1-Tag2 mice expressing the T antigen (Tag) under control of the rat insulin promoter (RIP), pancreatic β-cell cancer progression originates from Tag-induced inactivation of p53 and incomplete inhibition of the p16Ink4a/Rb pathway. In previous studies, we showed that Tag-specific, interferon-γ (IFNγ)- and tumor necrosis factor (TNF)-producing T helper 1 (TH1) cells doubled the survival of RIP1-Tag2 mice and inhibited β-cancer cell proliferation. Freshly isolated β-cancer cells are devoid of MHC class II, and MHC class II-restricted TH1 cells do not invade β-cancers but accumulate around the lesions. In the present work, we therefore focussed our research on the soluble TH1 cytokines IFN-γ and TNF and investigated their direct influence on β-cancer cells *in vitro*.
We determined the effects of IFNγ and TNF on proliferation and cell cycle control of β-cancer cells by BrdU incorporation and flow cytometry, respectively. In addition, the influence of TH1 cytokines on the p16Ink4a/Rb pathway was analyzed by Western blot. Treatment of subconfluent β-cancer cells for 72 h with either IFNγ or TNF significantly decreased the proliferation rate by 63% or 27%, respectively. Flow cytometry further revealed that the cytokines suppressed the S phase and arrested part of the β-cancer cells in the G0/G1 phase of the cell cycle. In line with this, we detected a cytokine-induced upregulation of the inhibitor of cyclin-dependent kinases 4/6 p16Ink4a. This in turn was associated with hypophosphorylation of Rb at Ser795 thereby stabilizing Rb-mediated cell cycle control. To demonstrate causal relationship between Rb hypophosphorylation and cell cycle control in β-cancer cells, we silenced Rb by the use of shRNA. Indeed, first experiments show that successful downregulation of Rb enhanced β-cancer cell proliferation. Taken together, our data demonstrate that pro-inflammatory cytokines secreted by TH1 cells directly act on cancer cells by stabilizing Rb-mediated cell cycle control. This newly described, nontoxic mechanism may significantly contribute to tumor surveillance by the immune system.

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Cd271+ve drug tolerant stem like melanoma cells play a crucial role in resistance to BRAF inhibitors

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More than fifty percent of melanomas carry BRAF mutations and specific inhibitors, like Zelboraf® (Vemurafenib; PLX4032) have shown to be successful in the treatment of patients with advanced disease. The therapy exerts an initial response rate of over eighty percent a major concern however, is the loss of sensitivity leading to a relapse. We observed that exposure of BRAF mutant melanoma cells to PLX4032 initially led to cell death, long term exposure to the appearance of CD271 positive cells with characteristics of a stem cell-like population. These cells are drug tolerant and are proliferating at a low pace with high levels of ERK and AKT phosphorylation when compared to the parental cells. The expression of CD271 was drug induced discontinuation again resulted in the formation of the parent cells sensitive to PLX4032. Combining BRAF or MEK inhibitors with TGF beta 1 significantly reduced or even completely abrogated the emergence of CD271 positive cells. Mechanistically, increased cell death by this combination was due to the downregulation of AKT and STAT3 Tyr 705 signaling. To summarize, we identified drug tolerant cells expressing CD271, which might be crucial for BRAF mutant melanoma in acquiring drug resistance when treated with BRAF or MEK inhibitors.

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Notch4 mediated regulation of Slug and Twist1 in melanoma

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Notch signaling exerts both oncogenic and tumor-suppressive properties in solid tumors, which is context dependent. Epithelial-mesenchymal transition regulators like Slug and Twist1 are important for tumor progression. We investigated, whether Notch4 signaling affects protein and transcript levels of Slug and Twist1 in melanoma cell lines with consequences for cadherin levels. Lentiviral overexpression of the intracellular domain of Notch4 (NICD4) resulted in a decrease of Slug and Twist1 protein levels and real time PCR showed a decrease of RNA transcripts. Gel shift assays and ChIP assays demonstrated binding of the key regulator of Notch signaling CSL to consensus sites upstream the transcription start site of the Slug and Twist1 promoter. Co-immunoprecipitation assays showed binding of NICD4 to CSL. Overexpression of NICD4 led to drastic phenotypic changes in a melanoma cell line with spindle cell morphology (WM9), leading to cobblestone cells. Concomitantly, a decrease in *N*-cadherin and a profound increase in E-cadherin levels at mRNA levels and partially at protein levels were observed. Our data suggest that Notch4 is suppressing Slug and Twist1 expression in melanoma and leads to phenotypic changes with alterations in cadherin expression.

P265 (O25)

Oncogene and copy number analysis of a larger cohort of conjunctival melanoma

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Conjunctival melanoma is a rare but potentially deadly tumor of the eye. Despite effective local therapies, recurrence and metastasis remain a frequently encountered problem. Once the tumor has metastasized treatment options are limited and the prognosis is poor. To date, little is known of the genetic alterations responsible for the development of conjunctival melanomas.
In this study we genetically analyzed a total of 78 conjunctival melanoma samples, to our knowledge the largest cohort reported to date. An oncogene hotspot array was run on 38 samples, screening for a panel of known cancer relevant mutations. Additionally 29 samples were analyzed for genome wide copy number variations using CGH-arrays.
We identified recurrent mutations in BRAF in 23/78 (=29%) of samples. Additionally, we found NRAS mutations in 14/78 (=18%) of tumors. Surprisingly, no activating mutations in KIT were identified in overall 42 samples screened. The copy number profiles obtained were analyzed both independently as well as in groups defined by the driving oncogene mutation. A wide array of copy number alterations affecting multiple chromosomes was detected, with a pattern reminiscent of cutaneous, in particular mucosal melanoma, and differing significantly from posterior uveal melanoma.
In summary we detected NRAS or BRAF mutations in a mutually exclusive pattern in roughly half (47%) of conjunctival melanoma and a copy number profile affecting large areas of the genome. Both findings argue for conjunctival melanoma being entirely distinct from uveal melanoma and closely related to cutaneous melanoma. We believe conjunctival melanoma should be categorized both pathogenically as well as therapeutically as a variant of cutaneous melanoma, giving affected patients access to the many novel treatment options available for these tumors.

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Molecular mediators of the senescent phenotype

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Mutagenic agents, UV irradiation or oncogenes put cells under stress and damage their DNA, thereby contributing to the initiation of cancer. However, the organism possesses an intrinsic protection mechanism called stress-induced senescence which counteracts cancer development. This tumor suppressor mechanism is characterized by long-term growth arrest and chromatin-remodelling resulting in the formation of senescence-associated heterochromatin foci. In addition senescent cells show a distinct morphology consisting of cytoplasmic and nuclear enlargement, vacuolisation and an oval to round cell shape. In our current study we used different stress stimuli such as chemotherapeutic agents and the expression of oncogenes to induce senescence in primary human melanocytes and thoroughly analysed the morphological changes associated with the senescence response. Our data show that the senescence phenotype was caused by the rearrangement of actin and tubulin filaments that resulted in the reorganization of the cytoskeleton and the formation of stress

fibres. Furthermore, time-lapsed videomicroscopy revealed that cellular motility of senescent cells was significantly impaired. Interestingly protein levels of focal adhesion kinase (FAK) and paxillin, both members of the focal adhesion complex family, are increased and both proteins accumulate at focal adhesion plaques in senescent melanocytes. Our data show that senescent melanocytes have a reduced ability to migrate which might be caused by the morphological changes associated with senescence.

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Expression of potential epidermal stem cell markers in Basal Cell Carcinoma (BCC) and tumours of skin appendages

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Stem cells are multi-potent cells that maintain the skin epidermis including skin appendages such as hair follicle, sebaceous glands and sweat glands. However it is still unclear if these cells also contribute to the formation of skin tumours arising from skin appendages. Therefore we tested 45 human basal cell carcinoma, including superficial, nodular, adenoid, infiltrating and sclerosing types and further 38 human tumours of skin appendages including 13 sebaceous adenoma and carcinoma, 20 eccrine sweat gland tumours and 5 pilomatricomas for expression of Lgr5, Lgr6, Lrig1, Cytokeratin 15 and Ephrin-B2 and compared these findings with unaffected human epidermis.

Overall we detected expression of stem cell markers except Lgr6 reported for epidermis and hair follicle in all tumours tested. Expression of Lgr5 and Lrig1 was generally lower expressed in more agressive tumours types such as sclerosing basal cell carcinoma and late poro-carcinoma than in less agressive superficial or nodular basal cell carcinoma or early poro-carcinoma and sebaceous gland tumours. In contrast, Ephrin-B2C was downregulated in infiltrating basal cell carcinoma including ulcus terebrans and progressing porocarcinoma. In conclusion we found expression of potential stem cell marker of epidermis and hair follicles that seems conserved and still expressed in skin tumours of appendages and BCCs. However during tumour progression those markers seemed to be more and more downregulated.

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Bax activation in course of its altered phosphorylation overcomes resistance to TRAIL-induced apoptosis in melanoma cells

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Resistance to TRAIL-induced apoptosis prevents its therapeutic use. Different strategies of TRAIL sensitization and a dependency on Bax have been described, but common principles of TRAIL resistance and the way of Bax activation remained poorly understood. Applying a melanoma model of TRAIL-sensitive and resistant cell lines, efficient sensitization for TRAIL is demonstrated for the kinase inhibitor BMS-345541. This effect was completely abrogated by Bax knockout or Bcl-2 overexpression, in accordance with the Bax dependency. Early loss of the mitochondrial membrane potential, release of cytochrome c and Smac clearly indicated an activation of mitochondrial apoptosis pathways. Of note, BMS-345541 alone resulted in early Bax activation, seen by conformational changes and Bax translocation. The synergistic effects resulted from Bid activation through TRAIL, which inhibits Bcl-2, and the activation of Bax through BMS-345541. The critical roles of Smac and Bid were clearly proven by siRNA knockdown. The way of Bax activation by BMS-345541 was unraveled by establishing a new assay for Bax activation. This showed reduction of the inactivating Bax phosphorylation at serin-184, while the activating Bax phosphorylation at threonin-167 was enhanced. Thus, modulation of Bax phosphorylation appeared as tightly related to TRAIL sensitivity/resistance in melanoma cells, and therapeutic strategies may be considered.

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Induction of apoptosis by the nonsteroidal anti-inflammatory drug (NSAID) diclofenac/HA is based on activation of intrinsic pathways and related to Bad upregulation as well as Mcl-1 and Bcl-w downregulation

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Actinic keratosis (AK) occurs on sun-exposed skin and is characterized by high prevalence and the risk to proceed to squamous cell carcinoma (SCC). Cyclooxygenase-2 (COX-2)-mediated prostaglandin E2 (PGE2) synthesis has been reported in AK and SCC, and the COX inhibitor diclofenac in hyaluronic acid (diclofenac/HA; Solaraze(TM)) was approved for AK therapy. In previous work we have shown moderate induction of apoptosis in 3/4 cutaneous SCC cell lines after treatment with diclofenac/HA as well as an enhancement of death ligand induced apoptosis. The mode of action of diclofenac/HA however, remained to be unravelled.

In the present study, diclofenac resulted in reduced PGE2 levels in apoptosis-sensitive cutaneous SCC cell lines (SCL-II, SCC-12, SCC-13) whereas no PGE2 and no COX-2 expression was detectable in a SCC cell line resistant to apoptosis induction (SCL-I). Activation of mitochondrial apoptosis pathways was evident in SCC cells due to loss of the mitochondrial membrane potential and release of the mitochondrial factors cytochrome c and AIF. Characteristic proapoptotic changes at the level of Bcl-2 proteins occurred in sensitive cells, as upregulation of Bad and downregulation of Mcl-1 and Bcl-w. In contrast Bad was already high, and Mcl-1 and Bcl-w were already low in resistant SCL-I, even without treatment, which may be explained by the lack of PGE2. An antiapoptotic downregulation of proapoptotic Bcl-2 proteins Noxa and Puma was however also seen in SCL-I, suggesting here pathways independent of COX-2. The regulations of Mcl-1 and Bad w ere also reproduced in SCC cells by the more selective COX-2 inhibitor celecoxib, thus further underlining the specific role of COX-2. The findings illuminate the mode of action of diclofenac/HA in SCC cells as well as principles of their resistance, which may allow further adaptation and improvement of the new therapy.

Miscellaneous

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Keratinocyte MMP-14 is dispensable during wound healing but modulates angiogenesis

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Proteolytic activity of matrix metalloproteinases is required for several cellular processes such as cell migration, release of factors/bioactive peptides and constant renewal of the extracellular environment. Among the MMPs, MMP-14 produced by keratinocytes has been suggested to be implicated in modulating cell migration and release of active fragments of extracellular matrix protein as laminin 332. It was previously reported that *in vitro* epidermal outgrowth from skin grafts of the MMP-14 knockout mice is altered. However, the causal role for keratinocyte MMP-14 could not be proven in this experimental system since MMP-14 was also depleted in the dermal compartment.

We have analyzed *in vitro* the migratory capacity of keratinocytes isolated from MMP-14-deficient. These experiments indicated a normal migratory capacity of these cells on collagen type I, IV and fibronectin but an enhanced migration on laminin 332. Thus, to address the cell-specific contribution of MMP-14 to migratory processes *in vivo*, we generated a cell specific MMP-14 mouse with inactivation of this enzyme in keratinocytes.

Keratinocyte-specific MMP-14-deficient animals displayed overall normal skin morphology and epidermal differentiation pattern. Upon wounding, repair in MMP-14ep−/− followed the same kinetics as in wild-type mice (MMP-14ep+/+). Moreover, microscopic analysis indicated that re-epithelialization, epidermal differentiation and granulation tissue formation was comparable in both mice genotypes. However, at day 14 post wounding, sustained angiogenesis was observed in MMP-14ep−/− mice in contrast to control mice. Interestingly, decreased levels of endostatin were detected in wound lysates of MMP-14ep−/− mice as well as in cultured primary keratinocytes. Taken together, these data indicate that MMP-14 expression in keratinocytes is dispensable for skin homeostasis and repair, but plays a crucial role in the epidermal-dermal crosstalk leading to modulation of vessel density.

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Activation and inhibition of blood coagulation by functional bio-macromolecules *in vitro* – a bimodal effect

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Introduction: Functional biomacromolecules (FBM) have a developing field of applications, e.g. biosensors, antimicrobial wound dressings, nano-coatings for medical applications and cosmetics. Furthermore, materials with advanced hemocompatibility have a great potential for intracorporeal use, such as for implants, or for the use in direct blood contact, e.g. dialysis tubes. Blood response to FBM was determined using different *in vitro* assays according to DIN norm EN ISO 10933-4 (2002, 2006). The study included the analysis of the effects on intrinsic and extrinsic activation pathways in whole blood as well as in plasma.

Materials & methods: Solutions of three different FBM (FMB1 – 3) in aqua dest. were used in this study. FBM1, -2 and -3 were differently equipped with polar headgroups. The time-dependent thrombin generation in normal plasma was determined via cleavage of a fluorogenic substrate by the serine protease thrombin using the Thrombin Generation Assay (Technothrombin TGA, Technoclone GmbH). The fluorescencent signal was measured with the SPECTROstar Omega (BMG LABTECH GmbH). Prothrombin time (PT) and activated partial thromboplastin time (aPTT) in plasma were measured in the coagulation analyzer MC1 (Greiner Biochemica GmbH). Blood coagulation was analyzed by incubation of the samples with recalcificated citrate blood. The free erythrocytes, not captured in a fibrin clot, were lysed with aqua dest. Then subsequently the hemoglobin content in the supernatant was measured at 540nm with the SPECTROstar Omega.

Results: FBM1 – 3 inhibited thrombin generation in a concentration- dependent manner in the TGA. Concentrations $\geq$5 g/ml completely prevented thrombin generation in human plasma. Thrombin activity, however, was not inhibited in this assay (data not shown). Furthermore, prothrombin- and activated partial thromboplastin time were prolonged in a concentration dependent manner. In the blood clotting assay a concentration-dependent bimodal effect was detected. Low FBM1-3 concentrations (10 g/ml) increased clot formation compared to control, whereas high concentrations ($\geq$100 g/ml) were shown to inhibit blood coagulation *in vitro*.

Conclusions: The general mechanisms of hemostatic action of different biomaterials are not yet fully understood. In this study, it was shown that high FBM concentrations inhibit intrinsic and extrinsic coagulation pathways in whole blood and in plasma samples. Furthermore, a bimodal effect of FBM in blood coagulation as well as a concentration-dependent inhibition of thrombin generation in the TGA were detected. Since no influence of FBM on thrombin activity in the TGA was found, FBM have the potential to be used e.g. as surface coatings for different medical equipments. Due to their properties high FBM concentrations could reduce the risk for thromboembolic events without inhibiting the physiological intravasal thrombin activity.

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Hemo- and biocompatibility evaluation of functional biomacromolecules *in vitro*

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Introduction: Functional biomacromolecules (FBM) offer an application range from technical to medical uses. Medical applications are for instance wound dressings, nano-coatings and biosensors. Therefore, biocompatibility testing of FBM is essential. In this study, three different FBM were tested. Cytotoxicity, activation of complement system and hemocompatibility were tested *in vitro*.

Materials and Methods: Solutions of FBM (FMBA, -B, -C) in aqua dest. were used in this study. The FBM differed in their chemical composition and structure. Cell Proliferation: Proliferation of HaCaT keratinocytes was determined after incubation of the cells with FBM dilutions up to 48 h using a luminometric ATP-assay (ATPlite(TM) M Kit, Perkin Elmer). The ATP dependent light generation was measured with a microplate laser luminometer (LUMIstar Galaxy, BMG LABTECH Ltd.). ATP is in proportion to the number of cells. Complement System: Complement activation of FBM has been tested with the Complement Convertase Assay (CCA, Haemoscan, Netherlands) by incubating FBM with plasma. Cleavage of a chromogenic substrate by C5 convertase could be verified by a change of the optical densitiy (OD). Signal could be determined with the SPECTROstar Omega (BMG Labtech). Hemocompatibility: Lyophilized plasma was recalcified with 0.2 M CaCl2 solution and the influence of FBM in different concentrations on the lag time and on the endpoint was determined.

Results: FBM B (<50 g/ml) exhibited the highest biocompatibility of the three FBM on HaCaT cells after 24 h incubation in the ATP-assay, with biocompatibility of FBM B > FBM C > FBM A. FBM A was most compatible relating to complement activation (up to 100 g/ml) and FBM C caused the highest complement activity in the CCA. A concentration dependent effect on the clotting time could be observed. FBM B <10 g/ml did not have substantial influence. FBM C [$\geq$10 g/ml] as well as FBM A [$\geq$5 g/ml] delayed clotting time. However, FBM A [$\geq$10 g/ml], FMB B [$\geq$50 g/ml], and FMB C [$\geq$50 g/ml] inhibited plasma coagulation completely.

Conclusions: In this study, three different FBM were tested. It was shown, that all three FBM activated C5 convertase *in vitro* depending on the amount of FBM bound to the microplate surface as well as on the chemical structure. Furthermore, all three FBM exhibited concentration-dependent effects on HaCaT-proliferation but were found to be biocompatible up to concentrations of <50 g/ml *in vitro*. In summary, investigations showed that FBM B is the most biocompatible FBM in all categories tested, although it had concentration-dependent limitation in the coagulometric clotting test (<10 g/ml). However, inhibition of coagulation pathways could be a positive aspect for using FBM as coating for e.g. central venous catheters.

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Complement activation by functional biomacromolecules *in vitro*

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Introduction: Functional biomacromolecules (FBM) are used for different medical applications, e.g. wound dressings, nano-coatings and biosensors. Therefore, hemocompatibility testing of FBM is of

great importance for an intracorporeal use with direct blood contact. The complement system protects the human organism against microbial infections by opsonization of foreign material. Therefore, phagocytes can recognize and eliminate pathogens. Hence, FBM's for medical applications should provide inert characteristics relating to the complement system. In this study, the Complement Convertase Assay (Haemoscan, The Netherlands) for solid biomaterials was adapted for soluble FBM. Activation of C5 convertase was determined in plasma.
Materials and Methods: Solutions of four FBM in aqua dest. were used in this study. The four FBM differed in their chemical composition and structure. The Complement Convertase Assay (Haemoscan, The Netherlands) detects the C5 Convertase activity by cleavage of a chromogenic substrate which mediates a change in the optical density. The absorbance was measured with the SPECTROstar Omega after 24 h at 405 nm (BMG LABTECH GmbH, Germany). A high binding microplate (Greiner, Germany) was coated with FBM film by drying for 90 min at 37°C. Reaction with ninhydrin solution was used to determine the FBM film. Plasma was incubated for 1 h at 37°C and washed afterwards to eliminate unbound complement factors. There was another attempt to test the FBM in their fluid phase by incubating the fluid FBM with plasma without coating the microplate before.
Results: High binding microplates (Greiner, 96 well) were coated with FBM in different concentrations. The four FBM tested varied in chemical modification of their functional groups. Differences in C5 convertase activity were observed after plasma incubation with the four FBM coated on the bottom of a microplate. In contrast, direct incubation with FBM solutions did not lead to C5 convertase activation except for one FBM, which showed a weak activation of complement at a concentration of 100 g/ml.
Conclusions: In this study, four different FBM were tested with regard to complement activation. It was shown, that C5 convertase was activated depending on FBM concentration bound on the microplate surface and their chemical structure. FBM < 50 g:ml did not activate C5 convertase *in vitro*. These results suggest that low FBM concentrations could be used in medical applications. It was shown, that activation of C5 convertase by film-forming biomacromolecules can be distinguished in the CCA *in vitro*. FBM in solution could not be discriminated according to concentration and structure at the same level as in the coating assay. Therefore, these biomacromolecules should be coated on a surface and not used in solution for this test.

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Evaluation of *in vitro*- hemocompatibility assays of functional biomacromolecules

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Introduction: The search for functional biomacromolecules (FBM) with possible applications as antimicrobial coatings for biosensors, wound dressings, medical textiles, or catheters and tubing systems is ongoing. Especially for an intracorporeal use with a direct blood contact it is essential to know the influence of FBM on blood coagulation. For that reason different *in vitro* assays according to DIN norm EN ISO 10933-4 (2002, 2006) were used in this study to evaluate hemocompatibility of FBM in whole blood as well as in plasma. The assays included the analysis of the effects on both intrinsic and extrinsic activation pathways.
Materials and Methods: Solutions of four different FBM in aqua dest. were used in this study. The FBM differed in their chemical composition and structure. The time-dependent thrombin generation in normal plasma was determined via cleavage of a fluorogenic substrate by the serine protease thrombin using the Thrombin Generation Assay (Technothrombin TGA, Technoclone GmbH). The fluorescencent signal was measured with the SPECTROstar Omega (BMG LABTECH GmbH). Prothrombin time (PT) and activated partial thromboplastin time (aPTT) in plasma were measured in the coagulation analyzer MC1 (Greiner Biochemica GmbH). Blood coagulation was analyzed by incubation of the samples with recalcificated citrate blood. The free erythrocytes, not captured in a fibrin clot, were lyzed with aqua dest. Subsequently, the hemoglobin content in the supernatant was measured at 540 nm with the SPECTROstar Omega.
Results: All tested FBM inhibited thrombin generation in a concentration-dependent manner in the TGA. Concentrations, that completely prevented thrombin generation in human plasma, differed with the chemical FBM structure. The thrombin activity, however, was not inhibited in this assay. Furthermore, it was shown that FBM prolonged both prothrombin- and activated partial thromboplastin time. The measurement of prolongation was also found to be dependent on concentration and chemical attribute. In the blood clotting assay a bimodal effect was detected for several FBM. Low FBM concentrations (10 g/ml) increased clot formation compared to control, whereas high concentrations ($\geq$ 100 g/ml) were shown to inhibit blood coagulation *in vitro*.
Conclusions: Since the normal mechanisms of blood coagulation and hemostatic events are not yet fully understood, it is even more difficult to evaluate the interactions between blood and biomaterials. In this study, different assays were used to determine the *in vitro* – hemocompatibility of FBM. It was shown that FBM affect thrombin generation, blood clotting, prothrombin- and activated partial thromboplastin time in a concentration- and structure-dependent manner. As such effects determine possible applications of FBM hemocompatibility assays provide great benefit in the development and assessment of FBM.

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Enhanced release of TGF-beta1 by MSCs rescues impaired wound healing in CD18-deficient mice

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Mutations in the gene encoding CD18, the common beta-chain of the beta2 integrin family, result in severe wound healing disturbances in human patients with leukocyte adhesion deficiency syndrome type 1 (LAD1). Previously, we have generated a CD18-deficient mouse strain, which revealed a severe delay in healing of full-thickness wounds. Using a full-thickness excisional wound model in CD18-deficient mice, we here demonstrate that adipose tissue derived mesenchymal stem cells (AT-MSCs) can be successfully used for cell-based therapy to support healing of difficult-to-treat chronic wounds. Notably, AT-MSCs injected around full-thickness wounds significantly accelerated wound healing at all stages in CD18-deficient mice compared to wild type control mice. AT-MSCs injection around CD18-deficient wounds fully restored decreased TGF-beta1 concentrations to that of wild type mice. The elevated TGF-beta1 in CD18-deficient wounds was mainly contributed by TGF-beta1 released by AT-MSCs up to day 7 post-wounding, eventually leading to augmented myofibroblast differentiation as indicated by increased expression of alpha-smooth muscle actin in the wound bed. These beneficial effects were significantly reduced when wounds were injected with TGF-beta1 silenced AT-MSCs. Taken together, these data suggest that local delivery of AT-MSCs may represent a promising tool to improve impaired healing in CD18-deficient chronic wounds via the release of TGF-beta1 and subsequent induction of myofibroblast-dependent wound contraction. Our results are of particular clinical relevance as decreased TGF-beta1 levels also constitute a major hallmark in the widely occurring chronic venous leg ulcers in human.

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Safe, efficient and painless delivery of MSCs from a chemically defined carrier accelerating murine full-thickness wounds

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Mesenchymal stem cells (MSCs) reveal promising potential for the treatment of chronic wounds. A major so far unmet challenge is the efficient, safe and painless delivery of MSCs to skin wounds. Previously, a surface carrier of medical-grade silicone produced by plasma polymerisation of acrylic acid (ppAAc) was developed, which successfully deliver various cells among them MSCs to deepithelialized dermal substrates *in vitro*. Herein we studied the potential of the ppAAc carrier to deliver human adipose tissue derived MSCs (AT-MSCs) to murine full-thickness excisional skin wounds *in vivo*. AT-MSCs cultured on ppAAc carriers for 4 days or longer did not change their cell surface marker expression profile, colony-forming, differentiation and immune suppression potential. Importantly, AT-MSCs delivered to murine wounds by ppAAc carrier significantly accelerated wound healing, similar to AT-MSCs delivered by intradermal injection. More than 80% of AT-MSCs could be transferred from carriers to wounds in 72 h. AT-MSCs were detectable in wounds for at least 5 days after wounding, by means of immunostaining against human-specific beta-2 microglobulin and PCR detection of human Alu DNA sequences in murine wound sites. Carrier delivered AT-MSCs were endowed with the capacity to down-modulate TNF-alpha-dependent inflammation, increase anti-inflammatory M2 macrophage numbers, and induce proliferation, angiogenesis and granulation tissue formation. In conclusion, the chemically defined ppAAc carrier is highly suited for efficient, safe and painless delivery of AT-MSCs to wounds eventually accelerating wound healing *in vivo*. This is of major clinical importance and a precondition for the delivery of AT-MSCs to successfully treat patients suffering from lipodermatosclerotic or otherwise fibrotic wounds where injection of MSCs is impossible.

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CDC42 activity regulates hematopoietic stem cell aging and rejuvenation

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The global trend in population aging is unprecedented – without parallel in human history. Aging of stem cells is, in combination with other factors, an underlying cause for aging associated diseases and tissue attrition with age. The identification of molecular mechanisms of stem cell aging is a first step towards developing rational approaches to attenuate stem cell aging. In the hematopoietic system, hematopoietic stem cell (HSC) aging is driven by both intrinsic and extrinsic factors and is linked to a decreased immune response, an increase in myeloid disease, late-onset anemia and a reduced regenerative capacity.
We previously demonstrated that the activity of the small Rho-GTPase Cdc42 is significantly increased in primitive hematopoietic cells as well as in other tissues of aged mice. Based on this observation we hypothesize that the increased activity of Cdc42 in aged HSCs may be causatively linked to cell-intrinsic aging of HSCs.
Cdc42 activity has been implicated in the regulation of cellular polarity, a phenotype thought to be critical for proper stem cell function. We therefore determined the polarity status of aged LT-HSCs by single cell immunofluorescence staining. Interestingly, young HSCs localize Cdc42, tubulin and several other polarity proteins in a highly asymmetric way, while aged HSCs are mainly apolar with respect to these proteins, implying apolarity as a novel phenotype and prospective marker of aged HSCs.
We then tested whether inhibition of the elevated Cdc42 activity in aged HSCs might attenuate phenotypes associated with HSC aging. Hence, we treated *ex vivo* sorted aged HSCs with a highly selective Cdc42 inhibitor to reduce Cdc42 activity in aged HSCs to the level measured in young cells. Decreasing Cdc42 activity reverts apolar aged HSCs into polar HSCs and rejuvenates aged HSCs functionally, as they differentiate *in vivo* more readily into lymphoid cells and maintain, compared to untreated aged HSCs, high regenerative capacity upon secondary transplants.
Therefore, our data imply a novel and critical mechanistic role for Cdc42 activity in the establishment of HSC polarity and in HSC aging and identify Cdc42 activity as a pharmacological target for rejuvenating cell intrinsic stem cell aging.

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Cell-tissue cross talk of SZ95 sebocytes with skin maintained *ex vivo* in a 3D co-culture model

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The maintenance of normal human sebocytes in organ culture and *in vitro* is extremely difficult and not reproducible, mainly because the cells are programmed to differentiate and undergo holocrine secretion, i.e cell membrane rupture and release of their content. Therefore, we developed a skin explant model, where skin specimens are co-cultured for 6 days with a monolayer cell culture of the immortalized human sebaceous gland cell line SZ95. Through model variation the molecular cross-talk between the cells and the skin specimens is possible both through direct cell-tissue and humoral contact. Interestingly the presence of SZ95 sebocytes in the culture system reduced the expression of IL-6 by the skin specimens, mainly at the the direct cell-tissue contact setting. In addition, DNA fragmentation (TUNEL technique) showed decreased apoptosis and enhanced Ki67 expression in basal epidermal keratinocytes of the skin specimens co-cultured with SZ95 sebocytes, indicating a normalising effect of SZ95 sebocytes in the *ex vivo* skin homeostasis. On the other hand, SZ95 sebocytes co-cultured with skin specimens showed increased lipid accumulation, suggesting that the co-culture settings promoted their differentiation. In conclusion, the aforementioned data underline a cross talk of human sebocytes and skin specimens under co-culture conditions but also a major role of sebocytes to skin homeostasis, proposing their addition to 3D skin models.

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Assessment of murine epidermal calcium distribution via 2-photon fluorescence lifetime imaging microscopy (2P-FLIM) in eczema and native Skin

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Calcium is a major player in regulation of keratinocyte differentiation and proliferation and is involved in establishing barrier function of skin. Atopic eczema is characterized by altered skin barrier function, keratinocyte differentiation and proliferation. To elucidate the putative role of alterations of calcium distribution in eczema, we investigated an OVA-based Atopic Dermatitis Mouse Model, in which we induced eczema of different severity. We here demonstrate at sub-cellular resolution the epidermal calcium distribution in ex-vivo samples via 2-Photon Fluorescence Lifetime Microscopy (2P-FLIM). 2P-FLIM enables to (i) directly correlate fluorophor-lifetimes to absolute Ca^{2+}-concentrations, (ii) optically penetrate into skin and (iii) circumvent effects like phototoxicity and photobleaching. By combining intensity (structure) and FLIM (Ca^{2+}) data, extra- and intracellular areas can be distinguished. In control skin, we find highest calcium concentrations at the transition between SG and SC which rapidly decrease in both directions, with lowest values at the Stratum basale (SB) and in the upper SC. Interestingly, in the granular cell layer intracellular Ca^{2+}-levels are higher than extracellular. In eczematous skin the overall Ca^{2+}-gradients are similar to control skin, but we

observe higher Ca^{2+}-concentrations especially at the border between SG and SC. Higher Ca^{2+}-concentrations are found extracellular as well as intracellular. The increase of extra- and intracellular Ca^{2+}-concentration is more pronounced in triple provoked murine skin compared to single provoked skin. However, even though the number of provocations shows a significant correlation with epidermal thickness and IgE-serum concentration, there is no significant correlation to Ca^{2+}-concentration, neither in the overall gradient, nor in the intracellular/extracellular concentrations in the stratum granulosum. This the first description of the alteration of extracellular and intracellular Ca^{2+}-levels in an atopic eczema mimicking model.

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Assessment of gaseous ammonia diffusing from the skin surface into the environment to investigate skin surface physiology – a pilot investigation

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In contrast to other molecules showing gas properties at standard physiological skin conditions, very little about the relation between gaseous ammonia diffusing from the skin surface into the environment and skin physiology is known. Therefore, the aim of the present study is to investigate the relation between these ammonia molecules and standard physiological characteristics such as gender specific differences, skin surface pH and transepidermal water loss.

Overall, 15 women and 15 men were included in the study. Ammonia diffusing from the skin surface was collected by laying the median right forearm for 2 min onto a petri dish of 6 cm diameter filled with 5 ml ion free water. With this procedure a small space of air between the skin surface and the water could be maintained allowing only gaseous ammonia and not ammonium ions to diffuse into the water. The amounts of ammonia collected were assessed using a spectrophotometric assay based on the Berthelot reaction. Skin surface pH was assessed using a glass electrode while transepidermal water loss was measured using an open chamber system. The statistical evaluation consisted of an explorative data analysis for identification of outliers and extreme values, calculation of mean values and standard deviation, and of calculation of correlation coefficients between ammonia values and skin surface pH and transepidermal water loss.

After the explorative data analysis the values of 13 women and 15 men were further evaluated. The average ammonia values assessed from the water reached 0.14 ± 0.08 mol in men and 0.08 ± 0.05 mol in women. Statistical comparison using t-test revealed a significant difference between men and women ($P = 0.026$). Correlation analysis revealed a significant correlation between ammonia and pH of the skin surface ($r = -0.582$; $P = 0.023$), which was even more significant after correlating pH with the reciprocal ammonia values ($r = 0.696$; $P < 0.001$). There was no significant correlation between ammonia values and transepidermal water loss.

The results obtained in the present study indicate gender specific differences of ammonia diffusing from the skin surface into the environment as well as a relation between ammonia and pH of the skin surface. With respect to the gender related findings differences in sweat gland activity between men and women might be an explanation as sweat contains significant amounts of ammonia and ammonium ions. With respect to the correlation between pH of the skin surface and ammonia with its inverse character it can be concluded that a lower pH of the upper skin might attract more ammonia into the skin and that this dominates the amounts diffusing from the skin surface into the environment. More studies are required to confirm the findings and to elucidate their underlying mechanisms and functional significance.

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Sculptra injections influence mRNA expression of genes related to the collagen metabolism in human skin

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The dermal filler Sculptra® is used successfully for minimal-invasive facial soft tissue augmentation since 1999. Poly-L-lactic acid (PLLA) is attributed to be the active ingredient. Clinical studies are available and the augmenting effect is as well documented as the management of side effects, such as granulomatous reactions. Despite its clinical effect, the underlying biochemical mechanisms have not been studied so far.

To reveal more details about the biological mechanism, 21 healthy female volunteers, aged 50–65, were subjected to a 20 months trial, receiving a total of four vials Sculptra (each 150 mg PLLA) to each ventral aspect of the upper arm. Injections were performed every 3 months. For more detailed observation over time, volunteers were divided into three groups at random, characterized by different biopsy time points. Prior to the first subcutaneous injection all volunteers were biopsied (baseline). Of each volunteer two more biopsies were taken 2 weeks after injection #1 and #4 (group A), injection #2 and 8 months after the last injection (group B), or injection #3 and 10 months after the last injection (group C). Biopsies were subjected to molecular biological examinations as well as to light microscopy. In Sculptra-treated tissue mRNA expression of collagen I and collagen III, tissue inhibitor of metalloproteinases 1 (TIMP1) and transforming growth factor ?1 (TGF?-1) were up-regulated over time. PLLA particles are birefringent under polarized light. Birefringent particles were found in sections of 52% of volunteers. In 95% of these sections inflammatory tissue response, fibrosis and even granulomatous reactions was visible upon light microscopy.

Furthermore, immunofluorescence microscopy shall confirm these findings on protein level. An increased synthesis of extracellular matrix proteins collagen I and collagen III could explain the augmenting effect of Sculptra. Increased TIMP1 synthesis would inhibit collagen degradation by matrix metalloproteinases. TGF?-1 may serve as a chemoattractant for fibroblasts, promote fibroblasts to differentiate to myofibroblasts and therefore stimulate collagen syntheses. Assumable is an encapsulation of PLLA particles according to a foreign body reaction, leading to an extracellular matrix deposition and finally to augmentation.

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In vitro evaluation of the debridement performance of a new debrider* compared to conventional cotton gauze

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Introduction: Wound debridement is a major challenge in the treatment of patients with chronic wounds, especially if wounds are covered with a firm fibrin slough. Here, conventional debridement methods relaying on cotton gauze may not be enough. However, surgical debridement requires trained personal, an operation theatre and, moreover, is often associated with severe pain for the patient. A new debrider* consisting of polyester monofilament fibres may present a novel, fast and almost painless option for wound debridement. Hence, we have investigated the debridement performance of this new debrider* *in vitro* and compared it to conventional cotton gauze**.

Materials and Methods: The wound debridement model used consists of glass plates coated with a thick protein crust, to imitate the wound slough, which is stained with haematoxylin. The debrider* and conventional cotton gauze** were used to debride/clean the glass plates under standardized conditions ($P = 0.067$ N/cm^2, $v = 1.6$ cm/s). Plate images were obtained before and after treatment. All images were processed using ImageJ 1.45m (NIH, Bethesda, MD, USA).

Results: It could be shown that the debrider* exhibited a significantly higher debridement/cleansing performance than conventional cotton gauze** *in vitro*. The debrider* was able to remove more protein slough from the glass surface compared to the cotton gauze** used, e.g. cotton gauze** reduced the clogged area about 10% while the debrider* removed more than 70% of the slough, respectively. Moreover, the debrider* was able to achieve a significant debridement/cleansing effect (area cleaned >70%) for at least four applications (one pad was used to clean four glass plates) while cotton gauze** quickly lost its efficacy from the first to the second glass plate.

Conclusions: It could be shown that the debridement performance of the new debrider* is significantly higher than that of conventional cotton gauze**. Moreover, the debrider* presents a non-invasive and therefore almost pain-free alternative to other techniques and can be performed without major expenditure in terms of time or materials. Hence, this new technique should provide a valuable tool in the treatment of patients with chronic wounds to improve the quality of life as well as to safe costs.

*Debrisoft®; Lohmann & Rauscher; **cotton gauze, Fuhrmann.

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Antibacterial and antifungal properties of a silver-coated fiber

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Introduction: Silver possesses a broad antimicrobial activity and has been used as antimicrobial agent for centuries. Hence, it can be expected that its use as functional antimicrobial coating of fibers and textile surfaces exhibits beneficial effects in household, industry and hospital settings. In this study, the antimicrobial effect of silver-coated polyamide fibers was evaluated according to the Japanese Industrial Standard (JIS L 1902: 2002) and by employing microplate-laser-nephelometry (NCCLS M27-A2).

Materials and Methods: The polyamide fiber samples were tested according to the JIS L 1902: 2002 for their antibacterial activity. In brief, 400 mg samples were inoculated with *S. aureus* (ATCC 6538), *S. epidermidis* (DSM 1798), *K. pneumoniae* (ATCC 4352), *P. aeruginosa* (DSM 1117), *C. albicans* (DSM 1386), *C. glabrata* (DSM 70614), or *A. fumigatus* (DSM 819) and incubated for 24 h at 37°C under aerobic conditions. For microplate-laser-nephelometry (MLN), extracts of the fiber samples (1 g:50 ml) were incubated with the microorganisms and growth curves were collected by measurement of the solution's turbidity (Nephelostar, BMG Labtech).

Results: Preliminary analysis according to the JIS L 1902: 2002 showed a strong antibacterial activity of the silver-coated fibers against all bacteria strains tested. Moreover, strong antifungal effects were confirmed against *C. albicans* (DSM 1386) and *C. glabrata* (DSM 70614). In addition, a significant antifungal activity against *A. fumigatus* (DSM 819) was observed. However, no antimicrobial effect of the fiber extracts were found using MLN.

Conclusions: Using various *in vitro* tests for the evaluation of the antimicrobial activity allows discrimination between different modes of action. Here, it was shown that the silver-coated fibers exhibit a significant to strong antimicrobial activity. However, it could be confirmed that silver is not directly released into the environment and that the antimicrobial activity of the silver-coated fiber is mediated by direct contact with the material.

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In vitro model for the evaluation of the effect of dressings used for negative pressure wound therapy (NPWT) on the tissue

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Introduction: NPWT is clinically effective in the treatment of chronic-stagnating wounds. Studies suggest that the positive effects result from cell recruitments to the wound site, where they contribute to granulation tissue formation. We showed that dressings used for NPWT exhibit different effects, cells especially grow into large-pored foams. Here, we look at the effects of dressings under NPWT on the tissue itself. Therefore, we used a tissue substitute to test the punch marking characteristics of dressings during NPWT *in vitro* employing optical profilometry to evaluate the results.

Materials and Methods: Dressing samples*+# were placed on the tissue substitute (10%gelatine, 10% milk powder) and connected to a vacuum pump by a vacuum seal. Experiments were carried out at −120 mmHg for 24 h under dry (no fluid-supply) and wet (additional fluid-supply) conditions. Embossing of the dressings into the tissue substitute was determined using optical profilometry.

Results: Measurement of the surface roughness was used for evaluating the dressings' effect on the tissue substitute. As expected, the large-pored PU-foam+ caused a higher irritation of the surface compared to the white foam*. However, combination of PU-foam+ and drainage-foil# reduced the effect of PU-foam+ on tissue substitute surface roughness. Surface roughness values after vacuum treatment were decreased about 30% in both, dry and wet conditions.

Conclusions: Using an *in vitro* model for NPWT combined with a gelatine/milk powder-based tissue substitute it could be shown that different dressings exhibit a distinct effect on the wound area. In the test series, it could be shown that the combination of large-pored PU-foam+ and drainage-foil# irritated the surface less than the PU-foam+ alone, achieving results comparable to white foam*. Hence, their combined application seems advantageous for negative pressure wound therapy.

* Ligasano/Ligamed, +Suprasorb® CNP foam/Lohmann & Rauscher, #Suprasorb® CNP drainage foil/Lohmann & Rauscher, Suprasorb® CNP-P1/Lohmann & Rauscher.

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In vitro evaluation of the influence of the pH on the antimicrobial activity of polihexanide and silver nitrate using microplate-laser-nephelometry

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Introduction: There is a shift towards higher pH-values in chronic wounds compared to acute wounds (called 'alkaline shift'). It was shown that the pH in chronic wounds most commonly has a range of 6.5–8.5. This alkalization is thought to be due to tissue necrosis and the presence of microorganisms. Hence, it is of interest to determine the pH influence on the efficacy of antiseptics. Here, we have used an experimental system based on microplate-laser-nephelometry to evaluate the pH influence on the activity of polihexanide and silver nitrate against Staphylococcus aureus and *Pseudomonas aeruginosa*.

Materials and Methods: Growth of *S. aureus* and *P. aeruginosa* was investigated by microplate-laser-nephelometry (NepheloSTAR, BMG Labtech), dose-response-curves were determined and IC50 concentrations for polihexanide and silver nitrate were calculated. The IC50 was used to evaluate the antimicrobial efficacy at different pH (5.0, 6.0, 7.0, 8.0, and 9.0).

Results: It was shown that low pH (5.0) effectively inhibits microbial growth. While no significant difference in the growth of *S. aureus* and *P. aeruginosa* was observed at pH 6.0 to 9.0, their progeny at pH 5.0 was found to be reduced to app. 10% of the control at pH 7.0. Furthermore, a significant influence of the pH on the efficacy of polihexanide and silver nitrate was found *in vitro*. *S. aureus* exhibited an increasing sensitivity against both antiseptics with rising pH. It could be shown that IC50 values of polihexanide significantly decreased from 0.5 g/ml (pH 6.0) to 0.06 g/ml (pH 9.0) and for silver nitrate from 2.1 g/ml (pH 6.0) to 0.5 g/ml (pH 9.0). *P. aeruginosa* displayed enhanced sensitivity only for polihexanide. Here, IC50 values decreased from 0.6 g/ml (pH 6.0) to 0.2 g/ml (pH 9.0). In

contrast, about 3-times more silver nitrate was necessary at higher pH to achieve a similar growth reduction of *P. aeruginosa in vitro*.
Conclusions: Employing microplate-laser-nephelometry it could be shown that polihexanide as well as silver nitrate possess a pH-dependent antimicrobial activity. Moreover, it was found that the influence of the pH on the efficacy of the antiseptics is different for Staphylococcus aureus and *Pseudomonas aeruginosa*.

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Functionalization of polyester with an antimicrobial coating

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Introduction: Functional antimicrobial coatings of textile surfaces are widely used in household, industry and hospital. Zinc oxide (ZnO) has a broad antimicrobial activity against gram-positive and gram-negative bacteria. In this study, the antibacterial activity of ZnO on a textile surface was evaluated according to the Japanese Industrial Standard (JIS L 1902: 2002). Moreover, extracts of the textiles were tested for antibacterial effects by microplate-laser-nephelometry (NCCLS M27-A2).
Materials and Methods: Polyester (PES) was used as textile surface for the coating with ZnO. Two different formulations of ZnO [ZnO(S) and ZnO(A)] were applied. Coatings were carried out with the two dispersions D1 and D2. The textile samples were tested according to the JIS L 1902: 2002 for their antibacterial activity. In brief, 400 mg textile sample was inoculated with Staphylococcus aureus (ATCC 6538) or *Klebsiella pneumoniae* (ATCC 4352). PES without coating was used as control. Samples were incubated for 24 h at 37°C under aerobic conditions. For microplate-laser-nephelometry (MLN), extracts of the textile samples (1 g:50 ml) were incubated with the bacteria and growth curves were collected by measurement of the solution's turbidity (Nephelostar, BMG Labtech).
Results: Analysis according to the JIS L 1902: 2002 showed a strong antimicrobial activity of ZnO(S) and ZnO(A) against Staphylococcus aureus (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). The two dispersions D1 and D2 had only marginal effects. Coating with ZnO(A) exhibited a higher washing stability compared to ZnO(S). Moreover, growth of Staphylococcus aureus and *Klebsiella pneumoniae* was significantly inhibited by extracts of ZnO(A) *in vitro* (IC50 = 0.02 and 0.03 mg/ml, respectively).
Conclusions: Polyester (PES) exhibits no intrinsic antibacterial activity but it can be easily functionalized with a zinc oxide coating for antimicrobial applications. In this study, a strong antimicrobial activity of PES with ZnO-coatings could be shown against gram-positive as well as gram-negative bacteria (Staphylococcus aureus and *Klebsiella pneumoniae*) *in vitro*. Moreover, it was found that the ZnO formulation A was superior to formulation S in regard to their washing stability.

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Comparison of the antimicrobial effect of two superabsorbent polymer-containing wound dressings *in vitro*

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Introduction: A variety of occlusive dressings for the treatment of chronic wounds is available, including films, foams, and gels, of diverse materials such as alginates, polyurethane, hyaluronic acid, or collagen. Not all of them are able to handle the excess amount of exudate of highly exuding wounds. Hence, dressings containing superabsorbent polymers (SAP) have been developed. SAP's are able to absorb a multiple amount of fluid of their own dry weight while keeping the wound environment moist. An additional inhibition of microbial growth would be beneficial. Important pathogens of nosocomial infections are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Candida albicans*. Hence, we have tested two SAP-containing wound dressings for their antibacterial and antifungal activity according to the JIS L 1902:2002 *in vitro*.
Materials and Methods: According to the JIS L 1902:2002, samples of 400 mg of the dressings*# were used for testing. The samples were incubated up to 24 h at 37°C under aerobic conditions with the pathogens. Additionally, effect on *P. aeruginosa* growth was investigated after a prolonged incubation period of 7 days.
Results: Both SAP-dressings exhibited a strong reduction (>3 log) of *P. aeruginosa*, *K. pneumoniae*, and *E. coli* after 24 h and were also able to significantly inhibit the growth of *S. aureus* and *C. albicans* (app. 2 log). Moreover, *P. aeruginosa* growth was completely inhibited over a period of 7 days. No significant differences were observed between the two dressings tested.
Conclusions: SAP-containing wound dressings show distinct antibacterial and antifungal properties. Their use should aid treatment of wound infections by entrapment of the microorganisms in the forming gel during uptake of wound exudate and the inhibition of microbial growth.
*Vliwasorb®/Lohmann & Rauscher, #Sorbion sachet/Sorbion.

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Phenotypic switching from M1 to M2 macrophages by mesenchymal stem cells improves wound healing in an iron-overload mouse model

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We have recently established an iron-overload mouse model which closely reflects main pathogenic aspects of chronic venous leg ulcers (CVU) in human. Similar to CVU, this mouse model showed impaired wound healing due to iron accumulation in wound infiltrating macrophages and unrestrained pro-inflammatory M1 macrophage activation with excessive release of TNFα and toxic reactive oxygen species. Mesenchymal stem cells (MSCs), in addition to their regenerative properties, were shown to exert immunomodulatory functions on innate and adaptive immune cells.
We here hypothesised that application of MSCs in wound margins may suppress the persistent activation of pro-inflammatory M1 macrophages and rescue the impaired wound healing in iron overloaded mice.
Macrophages exposed to Fe(III)-chloride/ascorbate and H2O2 *in vitro*, mimicking the Fenton reaction in wound associated iron overloaded macrophages *in vivo*, released high amounts of pro-inflammatory M1 cytokine TNFα. Notably, co-incubation of ®iron-macrophages with MSCs resulted in the significant suppression of macrophage-derived TNFα, while the release of the anti-inflammatory M2 cytokine IL-10 was significantly enhanced. Interestingly, injection of MSCs around full-thickness excisional wounds significantly accelerated wound healing of iron-overload mice when compared to control wounds injected with PBS or fibroblasts. This positive effect correlated with a reduced release of TNFα and the reduced expression of the M1 chemokine receptor CCR2 in iron overloaded wounds injected with MSCs when compared to control wounds, as assessed by Western blot analysis of wound lysates on days 2 and 5 after wounding. Using multi-parameter FACS acquisition and analysis of enzymatically digested wound tissue collected at day 5 after wounding, we found that injection of MSCs, but not of PBS or fibroblasts, dramatically suppressed the expression of the classical pro-inflammatory M1 markers TNFα, IL-12, CCR2 and Ly6C, while increasing the expression of anti-inflammatory M2 markers IL-10, IL-4Rα, CD204 and CD301 in iron-overloaded mice. These results strongly suggest that MSCs promote the phenotypic switching from pro-inflammatory M1 to tissue repair-promoting M2 macrophages, thus most likely accelerating the impaired wound healing in iron-overload mice.
Taken together, local application of MSCs may qualify as a promising therapy for macrophage-dominated inflammatory disorders such as CVU and other iron-overload conditions like multiple sclerosis and atherosclerosis.

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