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ALLERGY

P001 (OP01/03) | How autoimmunity fights with allergy: Impaired development of allergic contact dermatitis in type I diabetic mice

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Epidemiological studies revealed a decreased susceptibility to develop an allergic contact dermatitis (ACD) in individuals with autoimmune diseases like type I diabetes, psoriasis or rheumatoid arthritis compared to healthy individuals. However, the clinical relevance of this inverse correlation and the underlying immune mechanisms have not been evaluated so far. In our study, we wanted to confirm these results by analyzing the induction of a contact hypersensitivity (CHS) reaction, which resembles the ACD in humans, in non-obese diabetes (NOD) mice spontaneously developing an autoimmune insulin-dependent diabetes mellitus (IDDM). Female NOD mice were considered diabetic when blood glucose levels showed two consecutive readings above 250 mg/dL. In order to induce the CHS reaction, a $CD8^+$ Tc1-mediated cutaneous inflammation, the mice were epicutaneously sensitized with a contact sensitizer (e.g. the hapten TNCB), followed by an application of the hapten onto the ear to elicit the CHS reaction.

We compared the impact of the diabetic phenotype on the development of the CHS reaction in diabetic vs. non-diabetic NOD mice and also in non-obese resistant (NOR) animals as a further non-diabetic control strain. The experiments revealed that the existence of a clinically apparent diabetes in NOD mice protected from CHS development as demonstrated by a significantly reduced skin inflammation (diminished ear swelling, reduced cutaneous inflammatory infiltrate) as compared to non-diabetic NOD and NOR mice. In contrast, we did not observe an impaired hapten-specific $CD8^+$ Tc1 cell response (T cell proliferation, Tc1-cytokine (IFN-γ, IL-2) production) in skin-draining lymph nodes or the spleen of diabetic mice. However, increased levels of IL-10 were detected in diabetic mice with reduced CHS reactions as compared to non-diabetic NOD and NOR animals with an unaffected allergic skin inflammation. Blocking of the IL-10 effect by injection an anti-IL-10- receptor antibody during CHS induction completely restored the development of the cutaneous allergic inflammation in diabetic animals, indicating a functional role of IL-10 for the impaired CHS in diabetic mice.

In summary, our data indicate that the manifestation of an autoimmune disease like diabetes mellitus type I circumvents the development of CHS in mice and, therefore, confirmed the epidemiological data of a reduced susceptibility for ACD in patients suffering from autoimmune diseases. The identification of a novel link between the development of allergic and autoimmune diseases may result in new preventive strategies for inflammatory disorders.

P002 | Fibroblast MMP-14 is necessary for skin homeostasis and contributes to delayed-type hypersensitivity in mice

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MMP-14 is a membrane bound matrix metalloprotease that coordinates breakdown of extracellular matrix during tissue remodelling. To analyze the distinct function of fibroblast-derived MMP-14 in adult skin homeostasis, we generated mice with inducible deletion of MMP-14 in the dermal fibroblast (MMP-$14Sf^{-/-}$). To our surprise when deletion of MMP-14 was induced mice were smaller than control littermates. Moreover, the animals developed a fibrotic skin phenotype with increasing up to twofold thickness of the dermal connective tissue. Along with increased collagen type I, stiffness and tensile strength, while collagen cross-links were unaltered. In vitro, MMP-$14Sf^{-/-}$ fibroblast did not display significant enhancement of collagen de novo synthesis, but collagen type I accumulated as result of loss of collagenolysis by MMP-$14Sf^{-/-}$ fibroblasts. However, bleomycin-induced fibrosis in skin proceeded in a comparable manner in controls and MMP-$14Sf^{-/-}$, but resolution was impaired in MMP-$14Sf^{-/-}$. As we know that inflammatory reactions also depend on the infiltration of inflammatory cells into the tissues, we were interested to learn whether alteration of the dermal connective tissue could alter inflammatory reactions to external insults.

We therefore analyzed MMP-$14Sf^{-/-}$ mice response to croton-oil induced irritant contact dermatitis (ICD) and to a 1-fluoro-2,4-dinitrobenzene (DNFB)-induced delayed type hypersensitivity (DTH) models. We did not detect alterations in the ICD response in the absence of fibroblast-MMP-14. However, when we sensitized and challenged skin with DNFB, we found enhanced ear thickening in MMP-$14Sf^{-/-}$ mice compared to wild type mice. In addition, in the MMP-$14Sf^{-/-}$ mice at 48 and 96 hours post-challenge, we detected 3 and 5 folds increased CD3 and CD8 positive cells, respectively, than in wild type controls.

Taken together, our results have unravelled a crucial role for MMP-14 in the regulation of T-cell mediated inflammatory reactions.

P003 | Autologous serum therapy in autoreactive chronic spontaneous urticaria. A clinical evaluation and investigation of potential pathomechanisms

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Background: Chronic spontaneous urticaria (CSU) is characterized by spontaneously occurring itchy wheals, angioedema, or both. A certain proportion of autoreactive CSU (arCSU) patients show an immediate hypersensitivity type skin reaction after injection of their own serum as assessed by the autologous serum skin test (ASST). Recent studies have shown that repeated injections of autologous whole blood or autologous serum can be effective in the treatment of CSU. Autologous serum skin test (ASST)- positive CSU patients may show better responses than ASST-negative patients. The mechanisms of action are unknown, but have been postulated to involve tolerizing or desensitizing effects.

Objective: To investigate clinical outcomes and the mechanism of action in patients with autoreactive CSU treated with autologous serum.

Method: In the clinical part of this study 69 ASST-positive patients with mild to severe CSU were treated with weekly intramuscular injections of autologous serum for 8 weeks and followed up for another 12 weeks. During this period, disease activity was assessed with the Urticaria Activity Score (UAS7), Dermatology Life Quality Index (DLQI) and the use of on demand antihistamines. Moreover, the ASST was done at week 8 and week 20 and serum samples were obtained at several time points (9, 13 and 21 weeks).

Results: Therapy with autologous serum significantly reduced CSU disease activity ($P \leq .0001$), the use of antihistamines ($P \leq .05$) and quality of life impairment ($P \leq .05$) after 8 and 20 weeks. 30.4% of patients showed total response (UAS7≤6) in week 8 and the percentage increased to 47.8% in week 20. The majority of total responders in week 8 (n=21) reached their status at week 5 (n=14), however, three patients relapsed in week 12 and four patients in week 20, respectively. 33% and 36% of total responders turned ASST-negative in week 9 and 21, however, 33.3% of all patients turned ASST-negative in week 21. In all patients who turned ASST-negative at week 8 and 20, 53.84% and 43.75% of ASST-negative were total responders, respectively. The correlation analysis of change of UAS7 and DLQI from week 0 to week 21 was related positive (r^2=.38).

Conclusion and outlook: Autohemotherapy can reduce disease activity in CSU patients. Since there was no difference between responders and non-responders in the rate of becoming ASST-negative suggests that autohemotherapy does not work by acting on this pathomechanism. The analysis of the sera via basophil activation test and the measurement of total serum IgE levels against potential autoantigen candidates such as IL-24 by ELISA may reveal further details.

P004 | HSV-specific type 2 immune responses in patients with atopic dermatitis and history of eczema herpeticum

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Background: Eczema herpeticum (EH) is a disseminated severe HSV infection which occurs in a subset of patients with atopic dermatitis (ADEH). The role of T cells regarding the enhanced viral susceptibility is poorly understood.

Objective: We sought to characterize virus specific cytotoxic T (Tc) and T helper (Th) cells in 28 AD subjects, of which 14 had a history of EH.

Methods: HSV1- and influenza-specific Tc and Th cells were analyzed by means of reactive T cell lines, as well as the activation markers CD154 (CD40L) and CD137 (4-1BB), and MHC-tetramers. Thereby polarization surface markers and the cytokine response were assessed.

Results: Viral antigens led to a specific IFN-γ/Th1/Tc1-dominated immune response in healthy individuals. In AD and even more pronounced in ADEH$^+$ patients, HSV1- specific T cells as well as responsive T cell lines produced significantly more IL-4 but less IFN-γ. Polarization surface markers indicated an increase of both HSV1- specific CD4$^+$ Th2 and CD8$^+$ Tc2 which could be responsible for the IL-4 elevation. Interestingly, influenza-specific T cells of ADEH$^+$ patients displayed predominantly a shift from CD4$^+$ Th1 towards Th2, and fewer differences in the CD8$^+$ compartments.

Conclusion: This presumably inappropriate immune response to HSV1 may render patients with atopic dermatitis susceptible to EH.

ANGIOLOGY

P005 (OP02/02) | Stressed erythrocytes bind intravascular von Willebrand factor and promote microangiopathy

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Microangiopathy with subsequent organ damage represents a major complication in several diseases such as diabetes, connective tissue disease and sepsis. The mechanisms leading to vascular occlusion include the formation of ultra-large von Willebrand factor fibers (ULVWF) and platelet aggregation. To date, the contribution of erythrocytes to vascular occlusion is believed to be entirely secondary and passive.

In this study, we investigated the physical and molecular mechanisms underlying the interaction between stressed erythrocytes and ULVWF and its consequences on microcirculation and organ function under dynamic conditions. In response to stress, erythrocytes interacted strongly with VWF to initiate the formation of ULVWF/erythrocyte aggregates free of platelets. Aggregate formation was triggered by shear stress and depended on the external membrane expression of phosphatidylserine, annexin V and the A1 domain of VWF. VWF-erythrocyte adhesion was disrupted by heparin and the VWF-specific protease ADAMTS13. In an in vivo model of renal ischemia/reperfusion injury erythrocytes adhered to the peritubular capillaries of wild type but not VWF-deficient mice and the latter demonstrated less renal damage. In vivo imaging in mice confirmed the adhesion of stressed erythrocytes to the vessel wall. Moreover, enhanced eryptosis rates and an increased VWF binding were detected in blood samples from dialysis patients with chronic renal failure.

Our study demonstrates that following cell stress erythrocytes bind to ULVWF with marked consequences for vessel hemodynamics. The mechanism reported suggests that erythrocytes are crucial players in the pathogenesis of microangiopathies and renal damage; not by passively modulating but rather by actively disrupting hemodynamic conditions.

CELLULAR BIOLOGY

P006 | Skin pH and barrier recovery after application of buffers of different pH and composition

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pH and respective buffers are critical for the survival of cells. Every organ of the body has a distinct and tightly regulated pH where deviations may lead to diseases. We asked whether application of buffers of different pH und buffer capacity influences the skin's physiological pH and barrier recovery. Skin barrier was disrupted by tape-stripping on both flanks of hairless mice. Immediately thereafter phosphate, citrate and glycolate buffers with pH values from 4 to 9 compared to purified water were applied for 24 hours in patch test chambers. pH and TEWL as a marker for barrier recovery were monitored for 24 hours after removal of the test chamber. Also, skin samples were examined by immuno-histology with specific markers for epidermal proliferation and differentiation. We found that hairless mice of same breeding and housing conditions show a uniform skin pH of 5.8. After experimental barrier disruption pH increased 0.4-1.9 units; buffers with an acidic pH preserved a lower skin pH compared to buffers with a neutral or alkaline pH. Buffers with pH 4 were more effective than with pH 5.5. Every buffer delayed skin barrier repair compared to water in the order: pH 5.5 (lowest delay), pH 4 (phosphate/citrate), pH 4 (ammonium glycolate), pH 7 and pH 9 (highest delay). Interestingly, pH 7 and pH 9 buffers led to the highest increase in epidermal thickness, epidermal proliferation and inflammation. Buffers with pH 4 led to a smaller increase in epidermal proliferation and inflammation, but led to the same increase in differentiation markers compared to the pH 7 buffer. In conclusion, a pH 4 buffer has a stimulating effect on the skin, leading to moderate epidermal hyperproliferation, moderate inflammation and well-ordered epidermal differentiation for skin barrier repair. In contrast, the pH 7 and pH 9 buffers lead to a precipitously activation of epidermal repair mechanisms, pronounced inflammation, epidermal hyperproliferation and changes in differentiation. The chemical composition of the buffer also influenced the described effects, as seen by two different pH 4 buffers; however, these differences are smaller than the pH effects. We conclude showed that pH and the buffer system have an impact on barrier repair and inflammation in mice after skin barrier disruption. The results of this study might be used for development of novel topical products for treatment of dry skin and other indications characterized by a defect barrier.

P007 | Alpha-ketoglutarate suppress lineage differentiation and induces cell death in mesenchymal stromal precursors with dysfunctional mitochondria

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Increased concentrations of reactive oxygen species (ROS) originating from dysfunctional mitochondria contribute to diverse pathological conditions ranging from cancer, cardiomyopathy, and degenerative diseases to age-related disorders including skin aging. Little is known about how ROS impact on metabolism and fate of stromal precursor cells, despite recent evidence suggesting a ROS-dependent metabolic shift towards glycolysis. We here demonstrate that an increase in superoxide anions due to superoxide dismutase 2 (Sod2) deficiency in stromal precursor cells impair their osteogenic and adipogenic differentiation through fundamental changes in the global metabolite landscape as deduced by unbiased metabolomics. Our data identified a defective pyruvate and L-glutamine metabolism causing toxic accumulation of alpha-ketoglutarate in the Sod2 deficient stromal precursor cells as a major cause for their

reduced lineage differentiation. Alpha ketoglutarate accumulation led to enhanced nucleocytoplasmic vacuolation and chromatin condensation-mediated cell death in Sod2 deficient stromal precursors as a consequence of DNA damage, Hif-1α instability and reduced histone h3 (Lys27) acetylation. We thus uncovered a combination of previously unreported mechanism responsible for aging pathologies like skin aging and osteoporosis. In consequence our data can be exploited to therapeutically counteract mitochondrial pathologies commonly associated with aged individuals or caused by specific genetic mutations.

P008 (OP06/04) | Ceramide synthase 4 controls epidermal barrier homeostasis

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Ceramides are crucial for skin barrier function and their synthesis depends on ceramide synthases (CerS1-6), five of which are expressed in skin. Previously, using knockout mice, a crucial function was identified for CerS3 in the formation of a functional skin barrier (Jennemann et al, 2012), but it is less clear whether CerS3 is also the main producer of essential barrier ceramides during skin homeostasis. To address this question, we performed proteomic analysis and found that in adult mouse epidermis CerS4 and CerS5 are the predominant CerS isoforms. Whereas adult CerS5-deficient mice do not show any obvious signs of barrier dysfunction, 47 days old mice with an epidermal CerS4-deletion showed increased transepidermal water loss (TEWL). To understand how epidermal CerS4-deficiency leads to a disturbed barrier function, we first examined how CerS4 loss alters lipid composition in terminally differentiated keratinocytes and found an increase in the amount of key epidermal surface lipids, like cholesterol and ω-hydroxylated ultra-long chain (ULC)-ceramides, which precedes obvious barrier defects. These changes in the equilibrium of lipids of the cornified lipid envelope upon loss of CerS4 are accompanied by changes in terminal differentiation of keratinocytes, as reflected by an increase in filaggrin expression and processing. Morphologically, CerS4-loss induced lipid alterations lead to acanthosis and hyperkeratosis of the epidermis of adult mice. Together, our data show that an imbalance in epidermal surface lipid production disturbs epidermal barrier homeostasis and drives structural, functional and pathologically relevant skin barrier alterations in the epidermis and provide potential novel insight into lipid associated skin disorders.

Jennemann R, Rabionet M, Gorgas K, et al (2012). "Loss of ceramide synthase 3 causes lethal skin barrier disruption." Hum Mol Genet 21(3): 586-608.

P009 (OP05/05) | TGF-β1 signaling affects transcription factor NF-κB expression in an in vitro skin fibrosis model

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Skin fibrosis is a pathological process involving massive production of extracellular matrix (ECM) proteins, one typical characteristic of connective tissue diseases such as systemic sclerosis (SSc). As a central immunological regulator, the transcription factor NF-κB was shown to be involved in skin fibrosis; with respect to the five NF-κB proteins (p50, p52, p65, RelB and c-Rel), c-Rel has been implicated in pro-fibrotic functions in various organs while p65 impacts collagen I gene expression in dermal fibroblasts and p50 constitutes a genetic risk locus for SSc.

To further elucidate the function of NF-κB in skin fibrosis, expression of all five proteins was analyzed in human dermal BJ fibroblasts stimulated with TGF-β1 for different time points as fibrotic trigger. TGF-β stimulation induced fibrotic markers α-smooth muscle actin (α-SMA) and plasminogen activator inhibitor-1 (PAI-1) supporting the in vitro skin fibrosis model. Regarding NF-κB expression in response to TGF-β, p52 was reduced 72 hours after TGF-β stimulation while concomitantly, c-Rel was upregulated 48 hours after stimulation further supporting its pro-fibrotic impact. Subsequent c-Rel knockdown experiments were performed under homeostatic (w/ o TGF-β) and fibrotic (+TGF-β) conditions showing minor impacts on cell viability. Regarding expression of a small panel of fibrotic markers, c-Rel suppression did not affect gene expression level of α-SMA, PAI-1 and connective tissue growth factor (CTGF). Altogether, a better understanding of cellular signaling mechanisms associated with NF-κB and specifically with c-Rel in skin fibrosis may lead to new putative targets restricting the still uncontrollable fibrotic response in SSc and other connective tissue diseases.

P010 | In vitro assessment of the compatibility of application of PU foam and drainage foil during NPWT using different pumps

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Aim: NPWT has been advocated for virtually all kinds of acute and chronic wounds. Treatment is based on local negative pressure applied to the wound surface. NPWT is mainly carried out using open-cell polyurethane foams. It could be shown that cells especially show a significant tendency to grow into these foams which can be inhibited by application of a drainage foil without interfering with induction of cell migration. Hence, it is of interest to investigate if this combination is robust and workable with different vacuum pumps.

Methods: The drainage foil (Suprasorb® CNP drainage foil, Lohmann & Rauscher) was placed on fibroblast 3D-cultures in combination with large-pored PU foam dressing (CNP®foam, Lohmann & Rauscher). Assemblies were positioned in Petri dishes and sealed with air-tight film after medium supply and vacuum pumps (Suprasorb® CNP-P1, Lohmann & Rauscher; ATMOS® S 042 NPWT, Hartmann; RNASYS◊ GO, Smith & Nephew; ActiV.A.C., KCl) were connected. Experiments were carried out at −80 mmHg and −120 mmHg for 48 hours. Histology specimens were stained with haematoxylin/eosin and fibroblasts were detected using anti-vimentin antibodies. Cell viability and ingrowths of cells into samples was determined.

Results: Using the combination of drainage foil and PU foam samples during NPWT at −80 mmHg with different vacuum pumps led to the same cellular responses in vitro. With the PU foam dressing alone, cells did not stop at the pellicle edge but continued to migrate into the PU foam. In contrast, placement of a drainage foil between collagen pellicle and PU foam inhibited ingrowths of cells into the PU foam.

Conclusions: It could be shown that the combination of a drainage foil with a PU foam dressing for NPWT is workable with pumps from different manufacturers. The ingrowths of cells into large-pored foams can be inhibited in vitro by application of a drainage foil. In vivo this may prevent the disruption of newly formed tissue during dressing changes.

P011 | Application of non-adhering dressings during NPWT in vitro

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Aim: NPWT has been shown to be clinically effective in the treatment of chronic stagnating wounds. In vitro studies suggest that positive effects of NPWT result from the recruitment of cells to the wound site. It could be shown that the dressings used for NPWT exhibit different effects, cells especially show a significant tendency to grow into large-pored foams. We have used an in-vitro-model for NPWT to investigate the effects of the combination of the non-adhering dressings LP, DT, and MP with a large-pored PU foam dressing on fibroblasts.

Methods: The non-adhering dressing samples LP (Lomatuell®Pro, Lohmann & Rauscher), DT (Duratouch, Smith & Nephew), and MP (Mepitel®, Mölnlycke Health Care) were placed together with the PU foam dressing (Suprasorb® CNP Foam, Lohmann & Rauscher) on fibroblast 3D-cultures. The assembly was positioned in a 6-well-plate and sealed with a vacuum-applicator-lid (VAL). VALs were connected to medium supply and vacuum pump. Experiments were carried out at −80 mmHg for 48 hours. Histology specimens were stained with haematoxylin/eosin and fibroblasts were detected using anti-vimentin-antibodies. Cell viability and ingrowths of cells into samples was determined.

Results: Combination of the non-adhering dressings with the PU foam did not affect cells negatively and fibroblasts responded to subatmospheric pressure by migrating in direction of the applied vacuum. No distinct differences were observed in the application of LP, DT or MP during NPWT at −80 mmHg in vitro. In addition, no adverse effects on the structure of the non-adhering dressings were observed at microscopic level.

Conclusions: It could be shown that the combination of non-adhering dressings and PU foam demonstrates good cell compatibility and does not negatively affect cell viability. Moreover, combination of all non-adhering dressing and PU foam dressing samples allowed induction of fibroblast migration in direction of the applied vacuum during NPWT at −80 mmHg.

P012 | Herbal bitter drug Gentiana lutea improves the human skin barrier

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Human bitter taste receptors (TAS2Rs) are not only expressed in mucous epithelial cells of the tongue but also in various extragustatory organs, for example in epithelial cells of the colon, stomach and upper respiratory tract as well as in the skin (1). The signaling pathway of bitter compounds is dependent on the particular cell type.

Here we examined the impact of the bitter agent Gentiana lutea on human skin barrier formation. The skin barrier, also addressed as cornified envelope, consists of terminal differentiation proteins as well as epidermal lipids, mainly ceramides.

We analyzed the impact of Gentiana lutea extract (GE) and its isolated bitter compounds amarogentin and gentiopicroside on lipid synthesis. To address this issue, human primary keratinocytes (hPKs) were incubated for 2 days with GE or isolated bitter compounds. Nile red labeling revealed that GE increases lipid synthesis. As particular ceramides are important for maintaining epidermal lipid homeostasis and terminal differentiation (2) we investigated if GE also has an effect on ceramide synthesis. Therefore, we measured the expression of the key enzyme involved in ceramide metabolism, ceramide synthase 3 (CerS3) (3). Immunohistochemical staining showed that CerS3 expression was increased in GE-treated hPKs. CerS3 activation seems to be mediated by PPARγ and/or p38 MAPK signaling, because it could be reduced to background levels, by blocking the p38 MAPK or PPARγ pathway. Furthermore, GE and both amarogentin and gentiopicroside induced calcium influx and stimulated in hPKs the expression of differentiation proteins such as keratin 10, involucrin and transglutaminase.

Thus, GE not only enhances protein but also lipid synthesis in hPKs that is both essential for building an intact epidermal barrier.

This effect is partly mediated by amarogentin and gentiopicroside. GE might be used to improve skin disorders with an impaired epidermal barrier, e.g. very dry skin and atopic eczema.

Literature: 1) Ping Lu, View ORCID ProfileCheng-Hai Zhang, Lawrence M. Lifshitz, Ronghua ZhuGe. Extraoral bitter taste receptors in health and disease. JGP. 2017, 149 (2): 181.

2) Mullen, T.D.; Hannun, Y.A.; Obeid, L.M. Ceramide synthases at the centre of sphingolipid metabolism and biology. Biochem. J. 2012, 441, 789-802, doi:10.1042/ BJ20111626.3

3) Imokawa, G.; Abe, A.; Jin, K.; Higaki, Y.; Kawashima, M.; Hidano, A. Decreased level of ceramides in stratum corneum of atopic dermatitis: An etiologic factor in atopic dry skin? J. Invest. Dermatol. 1991, 96, 523-526.

P013 (OP03/06) | A20 is enhancing both TNF-induced apoptotic and necroptotic cell death by regulation of complex I and complex IIb formation in keratinocytes

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A20 is a zinc-finger protein with ubiquitin-regulatory functions and has an important role in the control of TNF signaling. A20 polymorphisms were associated with autoimmune pathologies and psoriasis. As a part of TNF complex I A20 inhibits NFkB activation which results in downregulation of inflammatory and apoptotic signaling. Cellular Inhibitors of Apoptosis Proteins (cIAPs) are known to inhibit both apoptotic and necroptotic TNF-mediated signaling by blocking the formation of another intracellular complex, namely the cytoplasmic complex IIb (Ripoptosome). We aimed to describe a possible role of A20 for TNF-induced cell death regulation in human and murine keratinocytes in the absence of cIAPs. Here we have either constitutively or inducibly overexpressed human or murine A20 protein in spontaneously immortalized human (HaCaT) cell line or immortalized murine keratinocytes, respectively. We demonstrate that upon A20 overexpression in HaCaT cell line canonical NF-kB activation was downregulated with concomitant upregulation of the non-canonical NF-kB signaling, as determined by NIK stabilization and p100 cleavage. Surprisingly, overexpression of A20 in either HaCaT keratinocytes or spontaneously immortalized murine keratinocytes did not result in protection from TNF-induced cell death, as expected, but, instead led to TNF-induced apoptosis. We have also detected an unmasked necroptotic cell death whenever the caspases were blocked. Both apoptotic and necroptotic cell death were significantly increased when cIAPs were absent. Moreover we were able to detect enhanced formation of TNF complex IIb (Ripoptosome) upon A20 overexpression in HaCaT cells, which can explain the increase of TNF-induced cell death. In contrast, the formation of TNF complex I was surprisingly suppressed upon A20 overexpression. Additionally cells with constitutively activated canonical NF-kB pathway were largely protected from A20-mediated cell death induced by TNF in presence of IAP antagonist. Taken together our data suggest that A20 inhibits canonical NF-kB activation, which leads to simplified formation of complex IIb, therefore enhancing both TNF-induced apoptotic and necroptotic cell death in keratinocytes.

P014 (OP02/04) | Role of fibroblast DPP4 on cutaneous repair and regeneration

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Cutaneous wound healing in mammals is associated with the formation of a scar which provides for a fast closure of the injured tissue but might lead to loss of function generating important health issues. The management of scars can be problematic and the goal of achieving scarless wound healing remains elusive. We have studied adult dermal fibroblasts (dFb), known to be responsible for the deposition of extracellular matrix (ECM) and the generation of scars. These cells are characterized by expression of Dipeptidyl peptidase-4 (DPP4, CD26) which through its activity is able to modulate cytokines and morphogens (such as Wnt family) around cells and thus influence their behavior. Previous publications show that DPP4 appears on dFb around embryonic day (E)16 coinciding with a lineage commitment of dFb and the onset of the scar phenotype. We show that expression of DPP4 is dynamically regulated on dFb during wound healing and is reduced at the moment dFb start presenting a pro-fibrotic phenotype and Wnt-activation required for scar formation. Pharmacological inhibition of DPP4 at early stages of healing of large wounds (1.5 × 1.5 cm) on the back of mice results in reduced thickness of the dermal layer but also in an abolition of hair follicle neogenesis (a hallmark of regeneration), whereas late inhibition results in unaltered dermal thickness but increased hair follicle neogenesis. This indicates that DPP4 possesses time specific effects on scar formation and skin regeneration. The targets of DPP4 at each stage of wound healing responsible for the regulation of scar formation and hair follicle neogenesis remain to be defined. The identification of which cytokines and members of Wnt family known to regulate skin regeneration are influenced by DPP4 activity in a temporal fashion will allow select molecular targets for prevention/treatment of scars and improve skin regeneration. Establishing when and how Wnt should be modulated for allowing complete regeneration of the skin but also reducing or inhibiting scar formation is of tremendous medical importance.

P015 (OP05/04) | A novel S100A8/A9 induced fingerprint of mesenchymal stem cells is associated with enhanced wound healing

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We here investigated whether the unique capacity of mesenchymal stem cells (MSCs) to re-establish tissue homeostasis depends on their potential to sense danger associated molecular pattern (DAMP) and to mount an adaptive response in the interest of tissue repair. Unexpectedly, after injection of MSCs pretreated with the calcium-binding heterodimeric DAMP protein S100A8/A9 into murine full thickness wounds, we observed a significant acceleration of healing even exceeding that after injection of wounds with non-treated MSCs as well as enhanced wound bed clearance. This correlates with a fundamental reprogramming of the transcriptome in S100A8/A9 treated MSCs as deduced from RNAseq approach and in-depth validated by means of RT-PCR and immunostaining of injected S100A8/A9 treated as opposed to injection of non-treated MSCs into the wound site. We uncovered a network of genes/proteins involved in proteolysis, enhancing macrophage phagocytosis and controlling inflammation, all contributing to a profound cleanup of the wound site. In parallel, increased expression of the previously - in the context of tissue repair - undescribed miR582 and genes boosting energy, as well as genes encoding specific extracellular matrix proteins are at least in part reminiscent of scar-reduced embryonic tissue repair. This novel MSC transcriptome not only underscores the concept that MSCs are endowed with the unique property to perfectly control multiple cell and tissue interactions like an orchestra conductor which coordinates all musicians in their complex interactions for the ultimate perfection of music, but also holds substantial promise to refine current MSC-based therapies for difficult-to-treat wounds and fibrotic conditions.

P016 | Inhibition of S-nitrosylation by trifluoperazine and impairment of the biological function of nitric oxide by calcium

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Trifluoperazine (TFP), a high potency antipsychotic drug used for the treatment of schizophrenia and schizophrenia-like conditions has further been used as analgesic in patients with neuropathic pain due to sickle cell anemia. Side effects of TFP include movement disorders and sedation. TFP is effective in part by reorganization of the cytoskeletal architecture, binding to and inhibiting of Ca^{2+}/calmodulin which affects numerous proteins in the erythrocytes, including the plasma membrane Ca^{2+} pump and eNOS. The present study explored whether TFP impacts the biological functions of erythrocytes, and if so, to test whether the effect is sensitive to Ca^{2+}, nitric oxide (NO) and S-nitrosylation. Eryptosis (programmed cell death of erythrocytes) was determined by flow cytometry, increase of cytoplasmic Ca^{2+} concentration by fluorescence microscopy and protein nitrosylation by fluorescence switch of the Bodipy-TMR/Sypro Ruby signal. Exposure of human erythrocytes to TFP significantly enhanced eryptosis, raised intracellular $[Ca^{2+}]i$, and decreased S nitrosylation. TFP-induced eryptosis was not affected by removal of extracellular Ca^{2+} alone, but was significantly inhibited by pre-treatment with the NO donor sodium nitroprusside (SNP). The inhibitory effect of SNP was significantly augmented by additional removal of extracellular Ca^{2+}. This points to antagonistic roles of Ca^{2+} and NO. Taken together, the results shown in this study deepen our knowledge about calcium- and nitric oxide-associated diseases, especially with respect to hematological or immunological disorders of the skin.

P017 | Intracellular redox changes alter tyrosinase protein stability and enable UVand MITF-independent skin color change

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Skin pigmentation is essential to balance UV-mediated vitamin d production and skin cancer risk. The microphthalmia-associated transcription factor (MITF) and its downstream genes, such as tyrosinase, and tyrosinase related protein 1 and 2, balance the pheo- to eumelanin ratio, hereby determining human skin, hair and eye color, as well as skin cancer risk. Adaptation of skin pigmentation has evolved during evolution. Today, changing skin color is desired for cosmetic and medical reasons. With a prevalence of over 80%, skin pigmentation disorders are considered as one of the most common medical problems, with currently very limited treatment options available. Our work describes the existence of a redox-dependent, but MITF- and UV-independent, skin pigmentation mechanism. Interestingly, skin color can be altered by modifying the activity of nicotinamide

nucleotide transhydrogenase (NNT), an enzyme being located in the inner membrane of the mitochondrion transferring reducing equivalents from NADH to NADPH, hereby changing the mitochondrial and melanosomal redox state. As NNT can be targeted by different small-molecule drugs, topical administration of these compounds resulted in a safe and fast change of human skin color. This redox-mediated alteration of skin pigmentation is based on a change of tyrosinase protein stability and modification of its degradation process. The newly identified NNT modifiers present a novel class of pigmentation drugs, which might be applied for cosmetic, medical and skin cancer prevention. In summary, the above-presented data highlights the existence of a distinct, so far unknown pigmentation pathway, offering novel therapeutic options for a large group of patients.

P018 | SPINK9 is a natural *Escherichia coli*-killing peptide in human skin

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Human skin is constantly exposed to microorganisms, but normally stays healthy. During the last years it became clear that our skin serves not only as a physical barrier against infection but also as a chemical barrier by production of antimicrobial peptides and proteins (AMP). Here we report the identification and characterization of a novel *E. coli*-cidal peptide isolated from human stratum corneum (SC) extracts. Using biochemical separation of high performance liquid chromatography (HPLC) followed by N-terminal sequencing and ESI-MS-analyses, we identified SPINK9 as a new member of *E. coli*-cidal AMP in the skin. SPINK9 was previously described as a Kallikrein-related peptidase 5-specific protease inhibitor. However, the N-terminal extension form with the first residue lysine exhibited highly killing activity against *E. coli* strains. Mutation analysis showed that the Kazal domain is necessary for the activity. Interestingly, we found SPINK9 interacts with SKP, an important periplasmic chaperone of *E. coli*. In conclusion we identify SPINK9 as a novel AMP in human skin. Our finding suggests a new chemical barrier role of SPINK9 other than the previously described function as a KLK5 inhibitor. The interaction between SPINK9 and SKP might contribute to new strategy of drug design.

P019 | Modeling of DNA damage induced senescence associated secretory phenotype

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Cellular senescence is a sort of permanent cell cycle arrest that results from exposure to various endogenous and exogenous stresses. Although cellular senescence seems to provide protection against malignant transformation, but in turn constitutes a fundamental cause of aging. Senescence in many instances can be accompanied by the release of some soluble factors from the senescent cells. These soluble factors collectively termed as senescence associated secretory phenotype (SASP). SASP is known to propagate senescence to the neighboring area as well as participate in the pathophysiology of several age associated disorders. Using published gene interaction data, we constructed a Boolean network model-based gene regulatory network of SASP. DNA damage induces cellular senescence and in turn the senescent cells secrete SASP mediators. Of these mediators, IL-6 and IL-8 are the most important ones, as these factors can induce and sustain the inflammatory state during aging as well as in many pathological conditions. Therefore, we simulated our Boolean model-based gene regulatory network of SASP in order to inhibit IL-6 and IL-8 expression in the condition of persistent DNA damage. The simulation predicts different in-silico knockouts that prevent key SASP-mediators. NF-B Essential Modulator (NEMO) or IKK-γ was one of the most promising among the predicted in-silico knockout candidates. Using in vitro experimental approaches, we validated some of the predicted candidates and further showed the importance of NEMO in the inhibition of IL-6 and IL-8 following DNA-damage in murine dermal fibroblasts. Therefore, using in-silico and in-vitro approaches we strengthen the speculated regulatory function of NF-B in the onset and maintenance of the SASP following DNA damage, giving an access to potential therapeutic targets for SASP-associated diseases.

PRESENTER: PALLAB MAITY

P020 | Generation of a Snap29 knockout in murine keratinocytes using CRISPR/Cas9

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Loss-of-function mutations in the SNAP29 gene have been discovered to be the cause of the rare autosomal-recessive neurocutaneous human CEDNIK (Cerebral Dysgenesis, Neuropathy, Ichthyosis, Keratoderma) syndrome. SNAP29 is a SNARE (Soluble NSF Attachment Protein (SNAP) Receptor) protein involved in membrane fusion and thereby epidermal differentiation as well as the formation of primary cilia and autophagy. Due to the small collective of available patients, a total as well as a keratinocyte-specific Snap29 knockout mouse line were created and resulted in epidermal hyperproliferation, abnormal keratinocyte differentiation and impaired epidermal barrier formation. In order to further examine the function of SNAP29, especially during epidermal differentiation, the utilization of keratinocytes is advisable. Since primary keratinocytes can only be passaged for a

limited amount of time, an immortal keratinocyte cell line harboring a Snap29 knockout would be beneficial for further research. Upon the different available genome editing tools, CRISPR/Cas9 is a very potent technique, allowing site-specific gene knockout. Currently, we are working on generating a CRISPR/Cas9 mediated Snap29 knockout in C5N immortalized murine keratinocytes.

P021 | 2A-DUB/Mysm1 regulates epidermal development in part by suppressing p53-mediated programs

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Development and homeostasis of the skin epidermis are governed by a complex network of sequence-specific transcription factors and epigenetic modifiers cooperatively regulating the subtle balance of progenitor cell self-renewal and terminal differentiation. To investigate the role of histone H2A deubiquitinase 2ADUB/ Mysm1 in the skin, we systematically analyzed expression, developmental functions, and potential interactions of this epigenetic regulator using Mysm1- deficient mice and skin-derived epidermal cells.

Strong Mysm1 expression detectable during murine epidermal development declined with age. Morphologically, skin of newborn and young adult Mysm1-deficient mice was atrophic with reduced thickness and cellularity of epidermis, dermis, and subcutis in context with altered barrier function compared with wild-type littermates. Skin atrophy correlated with reduced proliferation rates in Mysm1$^{-/-}$ epidermis and hair follicles relative to controls, as indicated by Ki-67 staining, and increased apoptosis in TUNEL assays. In addition, increases in DNA-damage marker γH2AX were detectable in Mysm1$^{-/-}$ skin. FACS analyses showed diminished fractions of α6-Integrinhigh^{+}CD34^{+} epidermal stem cells in Mysm1$^{-/-}$ skin correlating with significantly reduced colony formation of Mysm1$^{-/-}$ epidermal progenitors in vitro. In accord with an altered balance between epidermal self-renewal and differentiation, alterations in gene expression in the p63-Brg1/Satb1-Klf4 axis regulating transcription of epidermal differentiation complex genes were found at different developmental stages in Mysm1-deficient mice.

On the molecular level, we identified p53 as potential mediator of the defective Mysm1-deficient epidermal compartment where p53 protein levels were increased resulting in increased pro-apoptotic and anti-proliferative target gene expression. In p53$^{-/-}$Mysm1$^{-/-}$ double-deficient mice, significant recovery of skin atrophy was observed. Functional properties of Mysm1$^{-/-}$ developing epidermis were assessed by quantifying transepidermal water loss and skin hydration as well as by dye diffusion assays. In summary, this investigation uncovers a role for 2A-DUB/Mysm1 in suppression of p53-mediated inhibitory programs during epidermal development.

P022 | Combinatory treatment with IFNγ and triamcinolone - a new keloid treatment regimen?

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Impaired wound healing as well as imbalanced cell proliferation and extracellular matrix synthesis and degeneration can cause aberrant scarring. The most severe impact of such scarring on patients' life is stigmatization, physical restriction. Although a broad variety of treatment regimens including conservative approaches like compression therapy, invasive approaches including cryotherapy, surgical procedures and laser ablation as well as combinatorial approaches with e.g. glucocorticoids, chemotherapeutics and immunomodulators are used there is still a high recurrence rate of keloids. Aim of this study was to investigate which influence IFNγ and/or triamcinolone have on the proliferation, cell viability as well as collagen type I synthesis of healthy and keloidal fibroblasts.

The keloid fibroblast cell line (KF111) and normal fibroblasts were treated with different IFNγ and/or triamcinolone concentrations. Cell integrity, proliferation and collagen type I synthesis were time dependently analysed.

Our results show that during the observed time membrane integrity was neither influenced by IFNγ nor triamcinolone. Severe reduction of the proliferative potential of both cell species could be observed after treatment with IFNγ or triamcinolone for 2d. Whereas the combinatory treatment showed no additional effect in normal fibroblasts; a clear additional anti-proliferative effect could be observed in keloidal fibroblasts. When increasing the treatment duration to 4d the anti-proliferative effect of IFNγ and of the combinatory treatment regime was reduced in normal fibroblasts. In keloidal fibroblasts triamcinolone showed no longer an anti-proliferative effect whereas IFNγ and the combinatory treatment regime still showed severely reduced proliferation. Analysing the effect of both active agents and their combination on collagen type I synthesis revealed that in the used concentration range triamcinolone did not reduce collagen type I synthesis in normal fibroblasts, but reduced it in keloidal cells. IFNγ reduced in both cell species the collagen type I synthesis. In keloidal fibroblasts this collagen type I synthesis reduction could be intensified by combining both active agents.

The herein presented data suggest the combinatory application of IFNγ and triamcinolone as a promising therapy for hypertrophic scars.

P023 | Water-filtered near-infrared influences normal and keloidal fibroblasts differentially

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Impaired wound healing, imbalanced dermal cell proliferation, imbalanced synthesis and degradation of extracellular matrix are associated

with the development of hypertrophic scars. High recurrence rates, physical restriction as well as stigmatization are only some aspects influencing patients' life.

The panel of clinical applications of water-filtered near-infrared irradiation (wIRA) composed of near-infrared light (NIR) and a thermal component has increased in recent years. Due to the lack of conclusive discrimination between the thermal and the photomodulating NIR component, we decided to investigate the impact of NIR on dermal cells exposed to different temperatures. After focussing on cell morphology, catabolic and anabolic processes of extracellular matrix proteins in previous studies we investigated whether NIR influenced the induction of cell death in any way.

The keloid fibroblast cell line (KF111) and normal fibroblasts were kept for 56 minutes at temperatures between 37°C and 46°C in a water-bath connected to a peristaltic pump. The cultures were either kept light protected or exposed to 360 J/cm^2 NIR generated by a wIRA irradiator. Cytochrome C release, cleavage of pro-caspase3, DNA fragmentation as well as cell cycle were analysed at different times by ELISA, FACS or immunohistochemically.

Our results show that increased temperature induces cell death signalling in both herein examined cell types. Remarkably cytochrome C release and DNA fragmentation were higher in keloid fibroblasts compared to normal fibroblasts. The combinational treatment with NIR under hyperthermal conditions inhibited the pro-apoptotic influence of the high temperature; in detail reducing cytochrome C release, DNA fragmentation, activation of caspase-3 and lastly also reducing the sub G1 population of the analysed cultures. A clear difference between keloid and normal fibroblasts was evident when comparing cytochrome C release and DNA fragmentation after 4 hours. NIR inhibited cytochrome C release less pronounced in keloidal cells compared to normal fibroblasts. DNA fragmentation of the combined treated keloid cultures after 4 hours was even higher than the fragmentation in the light protected hyperthermally treated cultures. After 24 hours DNA fragmentation of the keloid fibroblasts after hyperthermal and NIR treatment was not significantly different to the exclusively thermal challenged cultures. In normal fibroblasts a significant difference was observed between the hyperthermal cultures and the combined treated cultures.

The herein presented data suggest NIR in combination with heat as a promising therapy for hypertrophic scars due to the observed pro-apoptotic effects.

P024 | Water-filtered near-infrared accelerates wound healing in vitro

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Imbalanced cell proliferation and migration are aspects leading to impaired wound healing and possibly also to chronic wounds. Treatment of such conditions is difficult and new treatment options are demanded. In this context the application of water filtered near-infrared (wIRA) composed of near-infrared light (NIR, 780-1400 nm) and a thermal component has increased in recent years. Aim of our study was to characterize the influence of NIR on dermal cells in in vitro induced wounds.

In confluent normal fibroblast monolayers artificial wounds/scratches were induced. Thereafter cultures were either kept light protected or were irradiated for 2 hours with NIR (154 mJ/cm^2) in a water-bath connected to a peristaltic pump, keeping the culture temperature constantly at 37°C. Wound closure was monitored for 24 hours with a live cell imaging system. Furthermore the binucleatic index and the cytokinesis index were determined. The liberation of the pro-inflammatory cytokines interleukin-6 and interleukin-8 was measured after 24 hours.

We could show that NIR significantly enhanced scratch closure and also increased both analysed indices. This indicates a pro-proliferative potential of the herein applied NIR treatment. Neither interlekin-6 nor interleukin-8 liberation was influenced by NIR, therefore we could show that the NIR dependent acceleration of the wound closure was not associated to these cytokines.

The application of NIR in wound treatment seems to be applicable due to increased fibroblast proliferation, lack of induction of interleukin-6 and interleukin-8, stimulation of cell migration and the low risk of thermal tissue damages.

P025 | Loss of proteostasis as a pathomechanism in premature ageing disease trichothiodystrophy

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The term, trichothiodystrophy (TTD) was first coined by Price et al in 1980 to describe patients with sulphur deficient brittle hair, which was then later characterized as a marker for this complex disease. TTD is a rare autosomal recessive, multisystem disease in which every organ of the body may be affected in particular the neuroectodermal tissues. Symptoms in particular ranging from delayed development, intellectual disability and cachexia to recurrent infections are manifested at an early age characterizing it as a progeroid syndrome. Approximately half of the patients with TTD suffer from a photosensitive form which is caused by mutations in three subunits (XPB, XPD and TTDA) of the basal transcription factor TFIIH. TFIIH is a multiprotein complex playing an essential role in initiation of transcription by RNA polymerases I and II and Nucleotide Excision Repair NER. TTD also serves as a disease model for accelerated ageing and its study could help in understanding physiological ageing. Preliminary data of our study on TTD cells show that the patients suffer from disturbed RNA

polymerase I transcription which further leads to disturbed ribosomal biogenesis. Further disturbance in the quality of ribosomes is indicated by the reduction in 18S rRNA along with reduced translational fidelity. Inaccurate translation leads to the increased amount of misfolded proteins which activates the Unfolded Protein Response (UPR) of a disturbed proteome. In return UPR represses RNA polymerase I transcription. Moreover in our study we also show that the use of chemical chaperones like TUDCA (tauroursodeoxycholic acid) can rescue ER stress thus restoring the disturbed RNA polymerase I transcription. These findings can overall help us in understanding the pathomechanism and also in implying possible treatments for this severe premature ageing syndrome.

P026 | Immunomodulatory effects of mesenchymal stem cells on macrophage activation

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

Mesenchymal stem cells (MSCs) are characterized by their ability to self-renew and their differentiation potential into multiple histogenetically distinct cell types. In addition they are endowed with the capacity to modulate immune cells, among them M1 macrophages, and in consequence dampen unrestrained inflammation at the wound site. To understand the molecular basis of MSCs and macrophages interactions that might be involved in down regulation of M1 activation by MSCs under inflammatory conditions, we employed qPCR and microarray analysis and specific ELISAs from co-cultured MSCs and macrophages. The transcriptome analysis showed that MSCs promote the conversion from M1, with low expression of the M1 markers TNF-α and IL-12p40, to M2 macrophages with increased expression of M2 markers like IL-10, IL-1RA and CD206. These findings were confirmed by qPCR and specific ELISAs. A comprehensive unbiased microarray analysis furthermore uncovered a variety of previously unreported molecular targets that might be involved in the MSCs control of the unrestrained activation of M1 macrophages. Among these new targets we identified osteopontin, an important multi-domain protein with chemotactic and inflammation modulating properties. Interestingly, osteopontin expression was significantly down-regulated by MSCs when co-cultured with pro-inflammatory M1 macrophages. Additionally we could verify that the macrophages activation states sensed by the MSCs are dependent on the direct cell contact and not only in the paracrine effect.

Our microarray analysis uncovered also leukemia inhibitory factor that was highly upregulated by the MSCs when in contact with M1 macrophages. Leukemia inhibitory factor is a multi-functional cytokine belonging to the IL-6 family. It is known to affect cell growth by inhibiting differentiation and it is induced under inflammatory stress. As it is upregulated by the MSCs after the contact with activated M1 macrophages, we are currently studying its role in the immunosuppressive action of the MSCs.

These results will help to gain knowledge on the conditions for MSCs employed for clinical use and may improve the effect of MSCs applied to the hostile proinflammatory microenvironment of chronic wounds.

P027 | Aberrant mTORC1 activity induces Stat3 and contributes to the psoriatic differentiation defect

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Psoriasis is a frequent and often severe inflammatory skin disease, presenting with red scaly plaques, which are caused by hyperproliferating keratinocytes that are unable to properly initiate the epidermal differentiation program. We previously found that aberrant activation of the Akt/mTORC1 cascade contributes to this defect. In healthy skin mTORC1 signaling is only active in the basal layer and contributes to the control of proliferation while preventing differentiation. When cells leave the proliferative compartment, mTOR signaling is switched off which promotes differentiation. However, under inflammatory conditions this switch is hijacked by inflammatory cytokines, which prevents proper differentiation. Beyond this model, it is currently unknown how mTORC1 mediates these effects on keratinocyte differentiation. We identified Stat3 as a putative downstream target of mTOR activity by phosphorylating Stat3 Ser727. Hyperactivation of Stat3 under inflammatory conditions blocked ordered differentiation in 2D keratinocytes cultures as well as in 3D epidermal models, which could be ameliorated by either mTORC1 or Stat3 inhibition. This shows a novel mechanism how inflammatory cytokines can mediate their physiological effect beyond the known activation of Janus kinases (Jaks). These results are especially interesting, as Jaks have recently drawn some attention by being novel therapeutic targets for small molecule inhibitors and suggest to also explore Stat3 inhibition as a therapeutic approach.

P028 | Suppression of autophagy compromises sweat secretion in aged mice

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Autophagy has been implicated in stress responses and cell differentiation in normal and diseased skin, however, the relative importance of autophagy in the various cell types of the skin is not fully understood. To investigate the role of autophagy in sweat glands of the mouse, the essential autophagy gene Atg7 was inactivated by Cre-lox recombination in keratin K14-positive precursor cells of epithelia and glands. The abundance of the autophagy substrate p62/sequestosome 1 was determined by immunofluorescence analysis. Sweat secretion was determined by the iodine-starch test on the soles of fully autophagy-competent control mice and mice carrying the epithelium-specific deletion of Atg7. The suppression of Atg7-dependent autophagy led to the accumulation of p62/sequestosome 1 (Sqstm1) in the secretory part of the sweat glands. Sweat secretion was normal in young Atg7f/f K14-Cre mice, but the number of active sweat glands was significantly reduced in Atg7f/f K14-Cre mice at an age of 10-14 months. These results suggest a previously unknown and possibly clinically relevant role of autophagy in the maintenance of sweat secretion during aging.

P029 | TNFAIP3 inhibits the induction of the antimicrobial peptide hBD-2 by Staphylococcus aureus in keratinocytes: Implications for atopic dermatitis

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The gram-positive bacterium Staphylococcus (S.) aureus is an abundant skin pathogen causing various skin infections. It is well known that the skin of atopic dermatitis patients is often infected with *S. aureus* but the underlying molecular mechanisms are still emerging. TNFAIP3 ("tumor necrosis factor alpha induced protein 3", A20) is a host regulator protein and involved in regulation of inflammatory processes by inhibition of the transcription factor NF-kappaB. Since we observed that *S. aureus* induced the expression of TNFAIP3 in human primary keratinocytes and in a 3D skin equivalent we sought to further define the role of TNFAIP3 in cutaneous innate defense, in particular in atopic dermatitis.

To this end we investigated the gene expression of TNFAIP3 in control biopsies and in biopsies of non-lesional and lesional atopic dermatitis skin. This revealed a significant increased TNFAIP3 gene expression in the lesional skin of atopic dermatitis skin as compared to healthy skin. In vitro experiments showed that *S. aureus* together with the atopic dermatitis associated TH2 cytokines IL-4 and IL-13 induced the expression of TNFAIP3 in primary keratinocytes. To further study the functional significance of TNFAIP3 we reduced the TNFAIP3 expression in human primary keratinocytes using TNFAIP3-specific siRNA. Keratinocytes with siRNA-mediated decreased TNFAIP3 expression showed an enhanced *S. aureus*-mediated activation of NF-kappaB. This was accompanied by an increased expression of human beta-defensin-2 (hBD-2), an antimicrobial peptide with the capacity to restrict the growth of *S. aureus* and to inhibit tissue-damaging proteases secreted by *S. aureus*. Based on our data we conclude that an enhanced expression of TNFAIP3 in atopic dermatitis skin contributes to a decreased *S. aureus*-mediated induction of hBD-2. A failure to adequately upregulate the expression of hBD-2 may trigger infection and inflammation associated with atopic dermatitis. Thus, the induction of TNFAIP3 in keratinocytes by *S. aureus* may represent a strategy of *S. aureus* to escape the action of host antimicrobial peptides such as hBD-2.

P030 | A DNA repair-independent pathomechanism in Cockayne syndrome and XP/CS

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Cockayne syndrome (CS) is a genetic disorder characterized by childhood onset of degenerative symptoms reminiscent of the aging body. Loss of subcutaneous fat, alopecia, cataracts, neurological degeneration and cachexia are the hallmark symptoms of the disease. These are accompanied by developmental delay, resulting in a severe phenotype and a short lifespan (approx. 12 years). CS is an autosomal recessive disorder. Mutations in five different genes are causal for CS (CSA, CSB, XPB, XPD or XPG), encoding for proteins involved in different stages of Nucleotide- Excision Repair (NER) mechanism, thus explaining the elevated UV-sensitivity of the patients.

Another function of CS proteins outside NER is in RNA polymerase I transcription. Here, we show that a disturbed RNA polymerase I transcription in CSA and CSB patient-derived cells is followed by a decreased translational accuracy of the ribosomes. This results in a high level of misfolded proteins and which are carbonylated due to increased ROS levels in CS cells. As a result, ER stress and unfolded protein response (UPR) are activated in CS cells and induce further repression of RNA polymerase I transcription. Additionally, we could show that UPR is activated in cells from patients with the more severe XP/CS, with mutations in XPB, XPD and XPG, indicating that our finding could represent a general mechanism in CS.

Our works elucidates that protein oxidation plays a central role in the previously described oxidative hypersensitivity of CS cells. This hypersensitivity can be overcame by using chemical chaperones such as tauroursodeoxycholic acid (TUDCA). Moreover, TUDCA can decrease ER stress and restore the deficient RNA polymerase I transcription and protein synthesis of CS cells.

P031 | Crucial role of the *S. epidermidis*-derived extracellular serine protease Esp for IL-1beta maturation in keratinocytes

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There is increasing evidence that skin commensals play a crucial role in cutaneous innate immunity. It is known that the abundant skin commensal Staphylococcus epidermidis (*S. epidermidis*) secrets the extracellular serine protease Esp. We have shown that *S. epidermidis*-derived Esp is able to process pro-IL-1beta proteolytically to the mature, biological active IL-1beta. To assess the physiological significance of these findings we first screened various clinical *S. epidermidis* isolates for their Esp-like capacity to process pro-IL-1beta to mature IL-1beta. We observed high differences in the ability of different *S. epidermidis* strains to process pro-IL-1beta. Although the majority of these strains exhibited IL-1beta processing activity, a few lacked any detectable IL-1beta processing activity indicating that these strains do not secrete sufficient Esp amounts to cleave pro-IL-1beta. Accordingly, HPLC and mass spectrometric analysis of *S. epidermidis* culture supernatants lacking IL-1beta processing activity did not reveal any detectable Esp. To gain further insight into the significance of Esp regarding IL-1beta maturation we treated human primary keratinocytes with Esp alone and in combination with different *S. epidermidis* supernatants. This revealed that supernatants lacking any detectable Esp activity did not induce a significant increase of secreted IL-1beta in contrast to *S. epidermidis* supernatants with Esp activity. Esp-reconstitution of *S. epidermidis* supernatants lacking any detectable Esp strongly induced the release of IL-1beta by the keratinocytes. Similar results were obtained using ex vivo skin explants. Together these data confirm that Esp mediates the processing of pro-IL-1beta released by *S. epidermidis*-treated keratinocytes. This in turn suggests that IL-1beta processing by abundant skin commensals such as *S. epidermidis* may represent an important component of cutaneous innate defense.

P032 (OP06/05) | Secretion of TGFβ1 depends on secretory autophagy

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TGFβ1 is a pleiotropic cytokine with cell type-specific effects modulating growth, survival and differentiation. Its activity has been shown to play key roles in tissue fibrosis and cancer. While signaling from different TGFβ receptors and extracellular activation of latent TGFβ are rather well understood, information on intracellular trafficking of the growth factor and its secretion is sparse, but has important biological and clinical implications.

Here we deciphered the molecular mechanism of TGFβ1 secretion by fibroblasts, the cell type that depends on autocrine TGFβ activity for differentiating into myofibroblasts. It is conserved across human and mice.

This work is based on our previous report that integrin-linked kinase (ILK) is required in fibroblasts for efficient secretion of TGFβ1, as ILK inactivation resulted in severely reduced TGFβ1 levels in fibroblast supernatants and concomitant intracellular accumulation. In vivo, mice with fibroblast-specific ablation of ILK displayed low myofibroblast counts in wound and fibrosis models with attenuated fibrotic responses (Blumbach et al, J Cell Sci 2010). This secretion defect was specific and did not affect bulk secretion, indicating that TGFβ1 is released through a regulated secretory pathway.

Several lines of evidence revealed impaired autophagy in ILK-deficient fibroblasts. To establish a potential link between autophagy and TGFβ1 secretion, autophagy incompetent fibroblasts and macrophages lacking essential factors such as ATG5, ATG7 or BECN-1 were cultured, which indeed showed virtual abrogation of TGFβ1 secretion. This result essentially rules out alternative secretory pathways in these cell types. Moreover, ultrastructural analysis then uncovered the presence of TGFβ1 inside autophagosomes in wild type fibroblasts. GRASP55, known for its role in maintaining Golgi morphology, is crucially involved in selecting TGFβ1 as cargo to be incorporated into autophagosomes. Our findings are reminiscent of the secretory autophagy pathway reported for the release of IL1β from macrophages. In line with those reports, TGFβ1 containing secretory autophagosomes are transported to the plasma membrane in a mechanism depending on RAB8A. It is unclear at present how TGFβ1 is released from its carriers to bind to extracellular matrix structures, and whether the other TGFβ isoforms or members of the large family of TGFβ proteins are also released by secretory autophagy. In-depth information on the molecular details of this pathway will offer a novel therapeutic target to modulate TGFβ release.

P033 | Biophysical characterization of neutrophil extracellular trap (NET) formation

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Introduction: Neutrophil extracellular traps (NETs) are fibril networks consisting of DNA and antimicrobial peptides expelled by neutrophil

granulocytes to entrap and kill pathogens. During their formation (NETosis), neutrophils massively rearrange their cell body. The nuclear chromatin expands and is finally released through the cell membrane into the extracellular space. As NETosis is also involved in the pathogenesis of several diseases, including cancer, vascular as well as autoimmune diseases, a deeper understanding of the fine-tuning of this fundamental immunological mechanism, would pave the way for new therapeutic strategies.

Methods: We used live cell imaging approaches such as confocal laser scanning microscopy (CLSM), reflection interference contrast microscopy (RICM), stimulated emission depletion (STED) nanoscopy and atomic force microscopy (AFM) to study NETosis on the single cell level. To this end, human neutrophils were activated with phorbol 12-myristate 13-acetate (PMA). Subsequently, the cell reorganization including chromatin decondensation, membrane and cytoskeleton rearrangement, as well as biophysical and mechanical properties during NETosis were analyzed. Additionally, inhibitors of enzymatic activity and cytoskeletal components were used to analyze the driving forces of this process.

Results: We identified three clearly distinct phases of NETosis: P1 is the active phase of NETosis characterized by a lobulated nucleus, high enzymatic activity and degradation of the cytoskeleton. P2 starts with the decondensation of chromatin and rupture of the nuclear envelope, which represents a "point of no return". The progression of P2 is dominated by passive mechanisms due to changes in cell material properties involving entropic swelling of chromatin, decrease in cell stiffness and significant cell rounding. At maximal circularity and minimal stiffness the cell membrane ruptures at a biomechanically predetermined breaking point and the NET is released into the extracellular space during P3.

Conclusions: We show that the formation of NETs is orchestrated by a complex interplay between biochemical signaling and changes in mechanical properties. Apart from its relevance for the understanding of NET release, this novel view on biological processes provides also general insights into cellular organization and dynamics and opens the door to novel pharmaceutical approaches.

P034 | Catch me if you can! -Novel immune escape mechanisms in melanoma metastases of the brain

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The immune system is one of the key modulator directing tumor growth and progression, but also therapy responsiveness. Based on recent reports it becomes clear that drug activity in the brain is not only directed by the drug susceptibility to penetrate the blood-brain-barrier, but is most likely modulated by the cellular composition of the brain-specific metastatic niche which modulates establishment and outgrowth of brain metastases. The immune microenvironment in brain metastases is active with a high density of tumor-infiltrating lymphocytes in certain patients and, therefore, may serve as a potential treatment target.

To study the reciprocal communication between tumor cells and the immunoactive infiltrate in the brain but also their impact on therapy efficacy, we were able to establish new preclinical models which allow for the first time to study homing specific mechanisms of brain-seeking melanoma cells in the presence of an intact immune system. Moreover, we identified a novel immune escape mechanism of melanoma cells penetrating the brain.

P035 (OP05/02) | Amino acid substitution in the C-terminal domain of Collagen XVII reduces laminin-332 interaction causing skin fragility with atrophic scarring

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The behavior of a cell depends on how its adhesion molecules interact with the cellular microenvironment. The hemidesmosomal component collagen XVII represents such an adhesion molecule that establishes dermal-epidermal connection in the skin. Although we and others have demonstrated that collagen XVII essentially contributes to cell adhesion and modulates keratinocyte directionality and proliferation during skin regeneration, only little is known about the involved molecular interactions. Here, we used keratinocytes from patients with junctional epidermolysis bullosa of late onset, which exclusively produce a collagen XVII mutant with a R1303Q mutation within its extracellular C-terminus. Although this mutant was normally expressed and showed no defects in membrane targeting, the keratinocytes were less adhesive, showed migratory defects and decreased clonogenic growth, while the expression rate of integrin subunits β1, β4 and of laminin-332 was normal. Moreover, expression of recombinant pR1303Q collagen XVII in human embryonic kidney (HEK) 293 cells caused increased apoptosis compared to HEK293 cells expressing wild-type collagen XVII. Since the R1303Q mutation is located within the predicted laminin-332 binding site of collagen XVII, we anticipated that it would alter collagen XVII-laminin-332 interactions. Indeed, the pR1303Q collagen XVII ectodomain showed decreased binding capability to laminin 332 and was less colocalized with pericellular laminin-332 molecules in immunofluorescence. Thus, aberrant collagen XVII-laminin-332 interaction results in reduced cell adhesion, destabilized cell motility as well as increased apoptosis, which in turn leads to blister formation, delayed wound healing and skin atrophy.

P036 | Extending the genomic knowledge of CTCL disease progression

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Introduction: Cutaneous T cell lymphomas (CTCLs) are non-Hodgkin lymphomas of skin homing T cells. The disease progression is greatly diverse between patients with around two thirds staying in an early disease stage with only limited skin patches or plaques. In one-third of the cases, the disease progresses to later stages with tumors, involvement of blood, lymph-node and viscerale organs which is generally associated with poor prognosis and fatal outcome. To date the reason for these different progressions remains unclear despite of various previous genomic studies (Damsky, 2016, Krejsgaard, 2017).

Methods: To investigate this issue and to guide future patient specific therapies we apply different molecular approaches to define possible markers to discern between different disease progressions, thereby analyzing the intrapatient and interpatient variations.

In detail, we use targeted high-throughput sequencing (HTS) to compare RNA and DNA samples from patients in indolent stages with samples from late-stage CTCL. DNA-Sequencing from FFPE samples with disease relevant genes from the Illumina TruSight One Panel has already started. Our special interest will be directed to genes and pathways notoriously mutated in CTCL (e.g. MAPK, Chromatin remodeling, JAK/ STAT, NFB, …). Also, T cell receptor clonality is known as an additional marker for CTCL and will be analyzed by HTS in parallel.

All sequencing data analysis is carried out with a GATK best-practices compliant pipeline to access SSNVs or SCNVs, and preconfigured pipelines are used to identify T cell receptor composition and relative abundance. The observed mutations will be validated by Sanger Sequencing and chromosomal aberrations will be verified by qPCR and ddPCR-assays.

Future prospects: Our aim is to develop a "CTCL directed Sequencing Panel" for diagnostic analysis from our data together with a pipeline for analysis which combines the detection of CTCL hot spot mutations and T-cell clonality by HTS in one workflow. We envision the usage of this workflow for application on tissue samples and liquid biopsies.

CHEMOKINES/CYTOKINES

P037 | TSLP production induced by skin irritation results from a functional synergism between PAR-2 and IL-1 pathways

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Background: Thymic stromal lymphopoietin (TSLP) is a pro-inflammatory cytokine and mainly derived from keratinocytes. Factors inducing TSLP and their mechanisms of action are incompletely defined. We previously reported that physical or chemical irritation rapidly induces TSLP in the skin and pinpointed this cytokine as a rapid responder to stressors elicited by deviation from homeostasis. We hypothesized that IL-1 and PAR-2 pathways promote TSLP expression upon skin perturbation.

Methods: Skin irritation was induced by tape stripping of mouse belly skin ex vivo and in vivo in the presence/absence of neutralizing antibodies or antagonists. In complementary experiments, murine skin explants and human keratinocytes (isolated from healthy donors) were stimulated with IL-1 and PAR-2 agonists. TSLP levels were measured by ELISA in explant or cell culture supernatant, mouse serum and skin lysates and TSLP mRNA quantitated by RT-qPCR. Chromatin immunoprecipitation (ChIP)was performed to examine the recruitment of NF-kappaB to the TSLP promoter.

Results: TSLP was induced in murine skin by tape stripping in a PAR-2 andIL-1 dependent manner. This finding was confirmed in skin biopsies stimulated with exogenous IL-1alpha plus PAR-2 agonist versus each stimulus alone. As with murine skin, a profound synergism between PAR-2 and IL-1 was observed in human keratinocytes. Mechanistically, we identified IL-1 and PAR-2 pathways acting on the TSLP promoter. PAR-2 activation together with IL-1 robustly augmented the recruitment of NF-kappaB to the TSLP promoter over IL-1 alone.

Conclusion: Skin barrier disruption results in concomitant activation of the IL-1 and the PAR-2 pathways, which act in concert to activate the TSLP promoter. The discovery of this concerted activity may have implications for a more flexible clinical management by selective targeting either pathway or both.

P038 | Analysis of anti-TNF-induced skin lesions reveals strong Th1 activation with some distinct immunological characteristics

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Background: Psoriasiform and eczematous eruptions are the most common dermatological adverse reactions linked to anti-TNF-alpha therapy. Yet, a detailed characterization of their immune phenotype is lacking.

Objectives: We sought to characterize anti-TNF-alpha induced inflammatory skin lesions on a histopathologic, cellular and molecular level, compared to psoriasis, eczema (atopic dermatitis), and healthy control skin.

Methods: Histopathologic evaluation, gene expression (quantitative RT-PCR) and computer-assisted immunohistologic studies (TissueFAXS) were performed on 19 skin biopsies from IBD (n=17) and rheumatoid arthritis (n=2) patients with new-onset inflammatory skin lesions during anti-TNF-alpha-therapy.

Results: While most biopsies showed a psoriasiform and/or spongiotic (eczematous) histopathologic architecture, these lesions were inconsistent with either psoriasis or eczema on a molecular level using an established CCL27/iNOS disease classifier. Despite some differences in immune skewing depending on the specific histopathologic reaction pattern, all anti-TNF-alpha-induced lesions showed strong IFN-gamma activation, at higher levels than in conventional psoriasis or eczema. IFN-gamma was most likely produced by CD3/CD4/Tbet-positive Th1 lymphocytes, but not CD3/CD8/Tbet-positive Tc1 lymphocytes or CD56/CD94-positive NK cells. While psoriasiform and spongiotic lesions on the trunk or extremities showed upregulation of the pro-inflammatory cytokines IL-36A, IL-36G, IL-19, IL-20 and the IFN-alpha-associated marker Mx1, psoriasiform scalp lesions that were clinically accompanied by patchy hair loss lacked increases of these mediators.

Conclusions: New-onset anti-TNF-alpha-induced eruptions previously classified as psoriasis or spongiotic dermatitis (eczema) exhibit a molecular profile that is different from either of these disorders.

P039 | Predictive models for the natural course of atopic dermatitis in childhood based on serum parameters and clinical attributes

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Atopic dermatitis (AD) is a common inflammatory skin disease, which usually starts in infancy. Most of the children have a remission during childhood, whereas about one third develops a chronic or chronic relapsing disease. However, it is not possible to predict the clinical course at an early stage. In this study, we investigated if serum parameters alone or in combination with clinical attributes might be useful to stratify infants for their individual risk of AD persistence.

128 patients (age 0-3 years) with confirmed diagnosis of AD were included in the study. Serum samples were taken between 2005 and 2011 and stored in the internal biobank. 29 cytokine and chemokine parameters, total IgE and specific IgE subclasses were determined. Information about family history, environmental influences, individual trigger factors, disease course and development of allergic comorbidities was collected retrospectively.

87% of the patients developed AD in the first year of life. 62.5% of the patients had a persistent disease until the age of seven years. Using statistical methods we established two logistic regression models. The first model is based on clinical attributes, such as premature birth, preventive creaming, individual trigger factors, and has a positive predictive value for disease persistence of 0.82 (AUC: 0.84). The second model is based on serum protein and IgE measurements and has a positive predictive value for disease persistence of 0.78 (AUC: 0.79). High concentrations of total IgE, RANTES, TIMP-2, IL12 and IFN-γ seem to be associated with AD persistence, whereas increased levels of TIMP-3, IL-10, G-CSF, IL-15 and IL-1ß were associated with AD remission. Both models can be evaluated separately and predicted probabilities can be combined for a more precise disease course prognosis. These results show that a combination of serum parameters and clinical attributes could be a useful tool to identify infants with high risk of lung disease persistence. These findings will need further validation on a larger cohort.

P040 | Role of CC-chemokine Receptor 6 (CCR6) and CC-chemokine Ligand 20 (CCL20) mediated immunosurveillance in malignant melanoma

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

We evaluated the homeostatic and inducible secretion of CCL20 by different murine and human melanoma cutaneous cell lines by enzyme-linked Immuno-absorbent assay (ELISA). Both, murine (B16, Ret) and human (A375, C32) melanoma cell lines are capable of secreting CCL20 upon stimulation with pro-inflammatory cytokines (i.e. TNF-á, IL-1á, IL-1 and TGF-) in vitro.

In order to determine the functional relevance of CCR6 on local tumor growth, B16 melanoma cells retrovirally transduced with a vector that constantly overexpresses CCL20 (B16-CCL20) were injected subcutaneously in wild type C57BL/6 (WT) and congenic CCR6-knockout (CCR6KO) mice. While animals in both groups developed local tumors, we observed a significantly reduced tumor growth in CCR6KO mice. By contrast, WT and CCR6KO control groups (injected with a B16 line that does not express CCL20) did not display differences in tumor growth rate. Our results suggest that CCL20 interactions in the microenvironment of cutaneous melanoma may be an essential factor for local tumor growth. Preliminary experiments have pointed out a possible autocrine pathway that would only B16 tumor growth in CCR6KO mice, although the precise mechanisms are still being investigated.

Current experimental approaches focus on the identification of the local immune infiltrate by means of Fluorescence activated cells shorting (FACS), expression of CCR6 and CCL20 in cutaneous melanoma of both WT and CCR6KO mice by means of real-time PCR.

P041 (OP03/01) | IL-17E promotes keratinocyte proliferation while interfering with proper keratinocyte differentiation

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Psoriasis is a frequent chronic recurrent inflammatory skin disease characterized by epidermal hyperproliferation and altered keratinocyte differentiation, combined with the presence of a massive immune cell infiltrate. As of now, neutralization of the IL-23/Th17 pathway, and in particular IL-17A, is the most effective therapy for the disease. We have recently demonstrated that IL-17E, an isoform of IL-17A, is overproduced by keratinocytes from psoriatic plaques when compared to non-lesional and normal skin. In this study, we aim at addressing the function of IL-17E in the epidermis.

IL-17E is expressed in normal and non-lesional skin in lamellar bodies of granular keratinocytes, as revealed by fractionation experiments and co-localization studies with specific lamellar bodies markers (Cathepsin D, Lamp1). In contrast in the psoriatic plaque, keratinocytes from all epidermal layers are positive for IL-17E. Using 3D epidermal models reconstituted from normal keratinocytes, we revealed that IL-17E is produced in response to inflammatory cytokines, including IL-17A and IL-22. Keratinocytes not only produce IL-17E, but also represent a target of the cytokine, given that they express both subunits of the IL-17E receptor (IL-17RB and IL-17RA) at protein and mRNA levels. Of interest, transcription of both subunits is enhanced along with the process of keratinocyte differentiation and upon stimulation with IFNγ. Mechanistically, IL-17E induced activation of PI3-K/Akt, mTOR and Erk pathway, leading to increased proliferation in 2D cultures. Consistently, increased numbers of Ki67 positive keratinocytes were measured in 3D epidermal models cultured in presence of IL-17E as well as in mice skin upon two consecutive intradermal injection of IL-17E. Despite the induction of proliferation, IL-17E did not induce acanthosis neither in the 3D epidermal model nor in vivo. Nonetheless, IL-17E interfered with keratinocyte differentiation, resulting in the up regulation of the early differentiation marker involucrin and down-regulation of the late differentiation marker filaggrin.

Together, our data demonstrate that IL-17E induces keratinocyte proliferation while leading to an aberrant differentiation, features characterizing the psoriatic plaque. Thus, we report here a novel and direct role of IL-17E in the alteration of the epidermal structure and function in humans.

P042 | IL-17RA signaling in keratinocytes is crucial for development of Imiquimod induced psoriasis-like dermatitis and for defense against Staphylococcus aureus

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IL-17A is the hallmark cytokine of Th17 cells and the funding member of the IL-17 family playing an important role in many autoimmune and inflammatory diseases such as psoriasis. The IL-17 response of Th17 cells and especially IL-17A-producing γδ T cells are crucial for mice to fight infections with Staphylococcus aureus. IL-17F has the greatest homology to IL-17A of the IL-17 family members. Moreover, IL-17A and IL-17F signal either as homo- or as heterodimers through a dimeric receptor composed of IL-17RA and IL-17RC. We used either IL-17RA full knockout or cell type specific IL-17RA deficient mice to delineate which cell type exactly needs to sense IL-17 in order to develop Imiquimod-induced dermatitis or to efficiently defend mice against *S. aureus* infection. Using this approach, we clearly define keratinocytes as the main cell population necessary to respond to IL-17 for Imiquimod-induced psoriasis-like disease and for the defense against *S. aureus* infection.

P043 | Glycosaminoglycan-based hydrogels capture inflammatory chemokines and rescue defective wound healing in mice

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Chronic wounds represent a challenge to conventional wound treatments. As a result, novel materials for the targeted treatment are urgently needed. Excessive production of inflammatory chemokines, establishing chemoattractant gradients, can cause chronic inflammation and therefore impair wound healing. Accordingly, capturing such chemokine signals using wound dressing materials may attenuate inflammation and thus may become a powerful treatment option for chronic wounds.

In here, a modular hydrogel based on star-shaped polyethylene glycol (starPEG) and derivatives of the glycosaminoglycan (GAG)

heparin was customized for maximal chemokine sequestration. The engineered hydrogel has been shown to effectively scavenge the pro-inflammatory chemokines MCP-1 (monocyte chemoattractant protein-1), IL-8 (interleukin-8), MIP-1α (macrophage inflammatory protein-1 alpha), and MIP-1β (macrophage inflammatory protein-1 beta) from wound fluids from patients suffering from chronic venous leg ulcers. Consequently, starPEG-GAG hydrogels considerably reduced the migratory activity of human monocytes and polymorphonuclear neutrophils using inflammatory conditioned medium and decreased the immune cell influx and inflammatory signaling in wound sites in a murine model of full-thickness excisional wounds (C57BL/6 mice). Finally, starPEGGAG hydrogels suppressed wound inflammation and improved granulation tissue formation, vascularization, and wound closure in an in vivo model of delayed wound healing (db/db mice).

Further testing of the promising starPEG-GAG materials may pave the way for a potential future application in human patients. Beyond that, the underlying concept is expected to be similarly applicable in the treatment of other disorders associated with pathologically enhanced inflammatory processes.

P044 | Intradermal ear injection of IL-17A and IL-36γ into mouse ears enables a model to investigate the cytokine network in Psoriasis

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Psoriasis affects 2-3% of the population in Western countries. The proinflammatory IL-17A, a member of the IL-17 cytokine family, plays a key role in the disease. Additionally, accumulating evidence has revealed that IL-36γ is given a high priority in the pathogenesis.

To understand more precisely the role of the IL-17A-IL-36 cytokine network in skin pathology, we used an ear injection model in our present study. Therefore, we injected IL-36γ and IL-17A alone and in combination into the ear pinnae of mice. After 4 days of consecutive treatment histological and immunohistological stainings were performed. The intradermal delivery of IL-17A and IL-36γ resulted in a significant increase of the ear thickness measured over time. Histological evaluation of IL-17A and IL-36γ treated skin showed a hyperparakeratosis, acanthosis with hypogranulosis, spongiosis, dermal edema and inflammatory infiltrate with many neutrophilic granulocytes. Mice that underwent injection with IL-36γ alone exhibited more eczematous-like skin changes, with mild epidermal hyperplasia with intact stratum granulosum and spongiosis. IL-17A on its own was not able to induce psoriasis-like changes in the mouse skin. Moreover, the expression of genes encoding antimicrobial peptides (AMPs), like mS100A8, mDEFB4 (ortholog of human HBD2), mS100A7A and mDEFB14 (ortholog of human HBD3) were upregulated after treatment with IL-17A and IL-36γ in combination. Similar effects were partially seen after the injection of IL-17A and IL-36γ alone, but the expression was weaker.

In conclusion, intradermal injection of IL-17A and IL-36γ in the ear pinnae of mice provides an in vivo model to investigate psoriasis. Our results strengthen the thesis that IL-17A and IL-36γ drive psoriatic inflammation via a synergistic interaction.

P045 | IL-17E participates in the recruitment of neutrophils in murine psoriasiform skin inflammation

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Psoriasis vulgaris is a common chronic recurrent immune-mediated skin disease, affecting approximately 2% of the population worldwide. We have recently found that IL-17E (i.e IL-25), a member of the IL-17 cytokine family, is over-expressed in lesional psoriatic skin when compared to non lesional and healthy donors. In vitro, IL-17E targets macrophages, leading to the production of IL-8 in a p38-dependent manner and to the ensuing recruitment of neutrophils in chemotaxis assays.

This study aims at understanding the role of IL-17E in neutrophil recruitment in a model of in vivo psoriasiform skin inflammation.

Daily intra-dermal injection of rmIL-17E in the back of BALB/c mice provoked a sustained and time-dependent inflammatory response compared to saline control group as assessed by immunohistological analysis. Although being rich in several immune cell types (T cells, neutrophils, eosinophils and macrophages), the inflammatory infiltrate was skewed towards a preferential recruitment of neutrophils in disfavor of T cells as revealed via a refined multi-parametric FACS approach. Consistently, the neutrophil chemokine CXCL1 was among the most abundantly expressed IL-17E-dependent genes in situ, as determined by nanostring analysis. In addition to the overexpression of chemokines, IL-17E induced the transcription of IFN type I related genes (e.g. IFIT2, IRF7), TNFα and amphiregulin, all of which are implicated in the pathogenesis of psoriasis. Noteworthy, the expression of IL-17E was enhanced following induction of psoriasiform inflammation in a murine tapestripping model. In this model, neutralization of IL-17E by intra-peritoneal injection of anti-IL-17E antibodies leads to significant reduction in the infiltration of neutrophils.

Consistent with these in vivo observations, the number of IL-17E$^+$ cells in human lesional skin correlated with the number of neutrophils, while being inversely proportional to the number of infiltrating T cells. Together, our data show that IL-17E favors the preferential recruitment of neutrophils in murine psoriasiform inflammation and define a novel role for IL-17E in psoriasis.

CLINICAL RESEARCH

P046 | Safety, tolerability, pharmacokinetics and efficacy of kappa-opioid receptor agonist WOL071-007-containing topical formulations in atopic dermatitis patients

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Previously, we have investigated the role of the newly developed kappa-opioid receptor agonist WOL071-007, belonging to the class of arylacetamide kappa agonists, during the progression of skin inflammation. Remarkable anti-inflammatory activity was detected in several murine models of dermatitis and psoriasis. Of note, the effects were mediated by kappa receptors as shown by pharmacological blockade and by experiments with KOR knockout mice.

Based on its preclinical profile WOL071-007 was selected as clinical candidate and tested in a first in human, single center, combined single/multiple ascending dose (Phase Ib), double blind, placebo controlled study to assess safety, tolerability, pharmacokinetics and local efficacy of 0.1%, 0.3% and 1% WOL071-007 containing formulations in AD patients.

The primary objectives of this study were to assess the safety of WOL071-007 by recording AEs as well as vital signs, the local tolerability of WOL071-007 as measured by change in local tolerability scores and the systemic safety of 3 concentrations of WOL071-007 (0.1%, 0.3% and 1%) in AD patients. The secondary objectives of this study were to assess the PK of WOL071-007 after topical administration in patients suffering from AD to a defined surface area of 10% of the body surface area. In addition efficacy of WOL071-007 was assessed by the change of local SCORAD.

The local tolerability of WOL071-007 and placebo was similar. At a dose of 1.0% under occlusive conditions, WOL071-007 penetrated not only into the epidermis but also into the circulation at concentrations that resulted in known substance-related pharmacological CNS effects that reached severe intensity due to a potentially increased drug penetration in eczematous skin under occlusive conditions. The study indicated a positive inter-individual effect of WOL071-007 over placebo on the IGADA and local SCORAD. However, this trend should be interpreted with caution due to the small sample sizes. Moreover, this difference is pointing to a systemic effect of WOL071-007 since the intra-individual comparison showed a very similar decrease of the local SCORAD for verum and placebo. Histology showed only a difference between placebo and verum at a concentration of 1.0%.

In summary, our results show that kappa-opioid receptor agonists may indeed be an attractive novel target to treat inflammatory and pruritic skin diseases. Additional studies with compounds exhibiting a higher peripheral selectivity need to be conducted in order to better separate peripheral anti-inflammatory effects from effects on the CNS.

P047 | An algorithm for the management of scabies mass outbreaks

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Background: Infestations with scabies mites are a global burden affecting individuals of all ages, classes and ethnicities. As poor sanitation and overcrowding favor the transmission of this highly contagious disease, epidemic outbreaks are frequently observed among displaced persons and asylum seekers. Due to the growing influx of refugees during the last years, public health authorities in host countries are frequently confronted with the challenge to treat individuals with diagnosed or suspected scabies promptly and effectively to avoid further spreading of the infection.

Objective: To establish a straightforward and efficient algorithm for rapid screening and treatment of large numbers of patients who are or are suspected of being infected with scabies mites.

Methods: 48 individuals from Syria (42% females, mean age 22.4 years, +/− 14.9 years), the majority of whom residing in local refugee hostels with confined living conditions were assigned to our dermatology department for screening and treatment of suspected scabies. According to their symptoms and signs patients were divided into 3 groups - (1) neither symptoms nor signs, (2) itch only, (3) itch and typical skin lesions - and treated with either a single dose of systemic ivermectin (group 1), 2 doses of systemic ivermectin at an interval of 7 days (group 2) or a combination of 2 doses of systemic ivermectin plus 2 applications of permethrin ointment at an interval of 7 days (group 3). Follow-ups were performed 4 weeks after initial treatments, individuals were instructed to avoid skin contact with itchy housemates and to decontaminate their textiles.

Results: All patients were treated according to the algorithm and followed-up after 4 weeks. None of the individuals of group 1 (n=32) had developed itch or skin lesions. In all individuals with initial itch only (group 2, n=5) its intensity had improved, 2 of which were free of symptoms. All individuals with additional signs of scabies (group 3, n=11) had improved itch, the skin lesions were completely resolved in 10/11 and partially in 1/11.

Conclusion: Our algorithm allows rapid treatment of large numbers of patients with scabies and their housemates. It proved to be both highly efficient for treatment of patients with diagnosed or suspected scabies as well as for prevention of asymptomatic individuals. Hence, this algorithm is well suited for the management of scabies mass outbreaks.

P048 | Clinical characteristics and treatment outcomes of patients with pyoderma gangrenosum - a single centre experience with 36 patients

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Background: Pyoderma gangrenosum (PG) is a rare non-infectious neutrophilic dermatosis of unclear aetiology. To date, no therapeutic guidelines have been established. We aimed to analyse the clinical characteristics and treatment outcomes of PG-patients treated between 2000-2016 at a single centre institution.

Methods: Thirty-six patients were enrolled in this retrospective study. We investigated the size of the ulcer, pain, treatment outcomes, quality of life and socio-economic parameters.

Results: Nine male (25%) and 27 female (75%) patients with an average of 50.9 19.0 years were analyzed. The legs were most commonly affected by PG (47.2%). 51.7% of patients had inflammatory bowel disease (34.6% ulcerative colitis (UC); 17.1% Morbus Crohn) of which two developed muco-cutaneous disease. In four cases (11.1%) the pathergy-phenomenon was noticed. Almost all patients were treated with corticosteroids (58.1% i.v.; 35.5% p.o.). Two thirds of the patients required inpatient care leading to at least partial remission in 91.3% of cases. Complete remission was found after a median of 16 weeks. 65.5% of the patients remained relapse-free. The mean pain-VAS score improved by 5.073.01 points at the end of therapy (scale 0-10). The average score reflecting mental distress was 8.11 (scale 0-10), the score representing limitations in everyday life was calculated with 8.4 points (scale 0-10).

Conclusion: Per os and i.v. glucocorticoids are equally effective for PG treatment. Regardless of the success of the initial therapy, 34.5% of patients suffered from relapses. Our analysis further emphasizes the severe impact of PG on everyday-life and mental wellbeing.

P049 | Differences in the response of dermatological symptoms and muscular strength after intravenous immunoglobulin therapy in dermatomyositis patients

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Introduction: Studies addressing the long-term efficacy of intravenous immunoglobulin (IVIG) treatment in dermatomyositis (DM) patients are sparse. Particularly, studies investigating differences in response patterns of cutaneous and muscular symptoms are lacking.

Methods: In this retrospective study, covering a follow-up period of up to 17 years, we analysed treatment outcomes of 15 DM patients. Investigated parameters included: Physicians Global Assessment (PGA), Cutaneous Dermatomyositis Area and Severity Index (CDASI), clinical characteristics and blood work.

Results: Four (27%) male and 11 (73%) female patients with an average age of 59.7 years were enrolled. All patients received additional immune-suppressive/immune modulating therapy, including glucocorticoids, azathioprine and mycophenolate mofetil.

PGA data revealed that muscular strength recovered to near baseline levels within the first 4 IVIG-cycles, with 11 patients (73%) experiencing at least moderate improvement after the first cycle. This observation was paralleled by the normalisation of serum CK-levels (average(initial)=1723 U/L; average(4cycles)=116 U/L, P=.024). Skin manifestations were recalcitrant: Four patients (27%) reached moderate improvement of their dermatological symptoms after the first IVIG cycle, eight patients (57%) had at least moderate improvement after 4 IVIG-cycles. Seven patients could be assessed after an average of 28.6 (range: 6-70) IVIG cycles, where improvement of skin symptoms in 4 of 7 patients was observed. Decreasing CDASI activity scores (average(initial)=17.4; average(follow-up)=5.1, P=.000) also highlight successful IVIG therapy for skin symptoms.

Conclusion: We conclude that combined IVIG and immune-suppressive/modulating treatment is effective recovering muscular strength in DM patients. Skin symptoms were improved in the majority of patients, but showed lower response rates.

P050 | Safety, tolerability, pharmacokinetics and efficacy of Glycopyrronium bromide-containing topical formulations in patients with primary axillary hyperhidrosis

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Hyperhidrosis is a dermatologic and neurologic disorder. It is a chronic and distressing condition involving increased production of sweat mostly due to overactivation of cholinergic signalling. As evidenced from the literature as well as prescription information there is a need for an effective and safe, topical medicinal product to treat primary, axillary hyperhidrosis.

Glycopyrronium bromide (GPB) is a well-known anti-cholinergic binding competitively to the muscarinic acetylcholine receptor (mAChR). Thus, it diminishes sweat production, the volume and free acidity of gastric secretions and controls excessive pharyngeal, tracheal, and bronchial secretions. An oil-in-water emulsion containing 0.5%, 1% and 2% GPB was tested in a first in human, single centre, combined single/multiple ascending dose (Phase Ib), double blind, placebo controlled study. Topical GPB or placebo was applied for 14 days followed by a follow up visit at day 21.

Primary objectives of this study (n=30) were to assess the safety and tolerability of topical GPB by recording AEs as well as local tolerability scores of 3 concentrations of GPB. Secondary objectives were

to assess PK and efficacy by gravimetric measurement as well as Hyperhidrosis Severity Score (HDSS; scale 1-4) after topical application of the different concentrations of GPB.

Local tolerability and systemic safety were similar to placebo for all concentrations tested. PK data show dose-dependency with highest concentrations measured after topical application of 2% GPB. Gravimetric measurements showed a significant difference for the pooled GPB data (all concentrations) compared to placebo (P=.002) in number of patients having a reduction in sweat production > 75% compared to baseline (87.7% vs. 48.1%). Topical application of GPB reduced sweating at day 14 compared to day 0 by 90% (0.5%), 78.5% (1%) and 92.2% (2%), respectively. However, HDSS at day 14 was reduced to 1 only following application of 1% or 2% topical GPB, but not for 0.5%.

In conclusion, the data from this phase 1b study show that the topical application of GPB in an oil-in-water emulsion is safe and well tolerated. Moreover, excellent efficacy is shown already in this small number of patients at low concentrations of GPB. To confirm efficacy and to show differences of the tested doses a phase 2b study will start beginning of next year.

P051 (OP03/05) | BRAF and MEK inhibitors change human immune cell phenotype and function; possible consequences for combination therapy

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BRAF and MEK inhibitors (BRAFi/MEKi), currently standard-treatment for patients with BRAFV600 mutated melanoma, are also explored as combination partner for various types of immunotherapy, notably checkpoint inhibitors, but also experimental therapies such as adoptive transfer of receptor-transfected T cells or dendritic cell (DC) vaccination. Since two BRAFi/MEKi combinations are approved, potential differences in their effects on the immune system would enable a rational choice for triple therapies. Therefore, we characterized the effects of the BRAFi/MEKi on T-cell activation and function, and DC phenotype and maturation in vitro.

We co-cultured TCR-transfected $CD8^+$ T cells and target cells and determined the cytokine secretion. We observed that the BRAFi vemurafenib (Vem) and dabrafenib (Dabra) had little effect, but the MEKi trametinib (Tram) and even more so cobimetinib (Cobi) clearly reduced cytokine production, while the functional avidity of T cells was not affected. Additionally, we assessed the influence on the functionality of chimeric antigen receptor (CAR)-transfected T cells. All BRAFi and MEKi reduced antigen-specific cytokine release as single agents, with Dabra having the mildest inhibitory effect. The combination Dabra+Tram had a clearly milder inhibitory effect than Vem+Cobi. A similar picture was observed for the activation markers CD25 and CD69. The cytolytic capacity of the CAR-T cells was strongly inhibited by Cobi (alone) and Vem+Cobi, whereas the other kinase inhibitors showed no effect. Therefore, the combination Dabra+Tram seems to be better suitable for combination with T-cell-based immunotherapy than Vem+Cobi.

Moreover, when looking at cytokine-cocktail-induced maturation of monocyte derived DCs, Vem (alone) caused the secretion of IL-8, IL-10, but also IL-12p70 during maturation, while Dabra (alone) and both MEKi had little effect as a single agent. When Vem was combined with Cobi, the IL-8- and IL-10-secretion was again reduced and the IL-12p70-secretion was abolished. An analysis of the maturation markers showed that CD25, CD70, CD80, CD83, and CCR7 expression were inhibited by Vem, while Dabra had much weaker effects. The MEKi seemed to increase CCR7 expression, and mainly repressed CD70 expression. Vem+Cobi could not rescue the effects of Vem alone, except for CCR7 expression, which was less affected. The combination of Dabra+Tram showed rather similar effects as Tram alone. In general, the combination Vem+Cobi had a stronger inhibitory effect on DC maturation than Dabra+Tram.

In conclusion, this work shows that the combination of Vem+Cobi affects different types of immune cells stronger than Dabra+Tram, indicating that the latter combination would be a better choice to apply together with immunotherapy.

SH and VE contributed equally, NS, LH, and JD share senor authorship.

P052 (OP04/02) | Application of circulating cell-free tumor DNA (ctDNA) profiles for monitoring metastatic melanoma progression under therapy

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Precision oncology is particularly seeking for novel disease and therapy monitoring technologies that are non-invasive and highly sensitive. Specifically, our project aims to establish blood-based assays that allow quantitative detection of ctDNA as a biomarker that corresponds to the tumor load in patients. ctDNA fragments are released from all parts of the tumors by apoptosis and necrosis, thus, it reflects the full spectrum of specific mutations of a systemically progressed tumor. With droplet digital PCR approach, we have analyzed 545 plasma samples from 77 stage III and IV melanoma patients with the BRAFV600E mutation, with mutations at the Q61 codon of the NRAS gene, and mutations in the promoter region of TERT gene. The patients received either MAPK-targeted treatment or immune checkpoint blockade. Additionally, plasma samples from

90 healthy donors were analyzed to test the positive and negative predictive values of our assays. ROC analyses showed over 90% AUC for all our assays. Our analyses revealed that increasing ctDNA levels were associated with disease progression from loco-regional (IIIB or IIIC) to systemic disease (IV) with $P<.05$. We evaluated our ctDNA assays with biostatistical methods, where ctDNA levels were correlated with treatment response and progression free survival. ctDNA levels during therapy corresponded to the radiologic tumor load from CT and MRI scans. Moreover, ctDNA levels often indicated disease progression earlier than the routine radiological scans. In brief, our results show the potential role of ctDNA measurement as a sensitive monitoring tool for the early assessment of disease progression and therapeutic response/resistance in melanoma patients.

P053 | Mapping of disease severity in patients with systemic sclerosis

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Systemic sclerosis (SS) is a severe autoimmune disease with a high mortality rate. Characterized by increased synthesis of collagen and damage to small blood vessels, first symptoms are thickening of the skin and poor blood flow to the fingers. Objective disease scoring is crucial for optimal disease monitoring and appropriate treatment. Most frequently the Rodnan skin score (RSS) is used as a primary or secondary outcome measure in clinical trials and daily routine. However, it is based on subjective assessment of the skin thickness and therefore does not represent an objective measurement tool. In this study, we aimed at testing the diagnostic potential of skin function measurements in SS. Twelve patients with conformed diagnosis SS were enrolled in the study. Skin fibrosis was assessed by conventional RSS and involvement of inner organs and serum inflammation parameters were determined. Four objective criteria, namely transepidermal water loss (TEWL), corneometry, pH and elasticity, were assessed at nine predefined sites of the body. Results were compared to patients with atopic dermatitis (n=10) and healthy subjects (n=7). Although SS patients varied in disease severity and levels of systemic inflammation, skin function measurements of SS patients represented the most homogeneous group at all sites of the body in contrast to atopic dermatitis patients and healthy volunteers. As expected skin elasticity was exclusively lowered in SS. Interestingly, we detected a decreased TEWL at the fingers and hands of patients with SS, indicating a link between impaired microcirculation and skin barrier, while skin humidity and skin pH of SS patients were not altered compared to controls. Our results demonstrate that skin function at the extremities are partially altered in SS patients. However, further studies of additional diagnostic tools are needed for mapping the disease progression more comprehensively and customizing treatment strategies accordingly.

P054 | Effect of intense pulsed light (IPL) plus radiofrequency (RF) on hidradenitis suppurativa

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Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease characterized by the development of multiple nodules/abscesses in inguinal and axillary/submammary regions, affecting roughly 1-2% of the population. Disease severity increases over time and can severely impact the quality of life. Treatment options are limited to topical treatment, intermittent administration of antibiotics, to adalimumab in severe cases. Recently several case reports have reported about a clinical benefit from treatment with radiofrequency (RF) or intense pulsed light (IPL) in acne vulgaris and hidradenitis suppurativa (HS). In the present study termed NICE (non-invasive combination therapy of IPL and RF in acne inversa) using the Laight® therapy, we investigated the effect of a combination of IPL plus RF for the treatment of HS.

In this monocentric, prospective, blinded, cross-over design study, patients were enrolled between 7/2014 and 3/2016. After an observation period of 3 months, all patients were randomized in a 1:1:1 fashion to one of the following treatment arms: 1. IPL plus RF, 2. IPL only and 3. RF only. All treatments were applied for 12 weeks. Thereafter, all patients received another course of 12 weeks of full IPL+RF treatment (cross-over). Biweekly treatment was applied to all body areas affected by HS. Every 3 months, disease activity as well as quality of life was documented. A total of 47 patients were enrolled. The group receiving treatment with IPL plus RF showed best treatment results, followed by those receiving RF only for the first 3 months. The overall improvements of the combination therapy in a modified Sartorius and a disease activity score were 20 and 16%, respectively. In addition, the DLQI improved from 16.1 to 8.9 points (-45%). Patients with Hurley grade I/II clearly benefitted more from this treatment modality as compared to patients with more severe disease (Hurley grade III), i.e. the overall improvement in the modified Sartorius was 22% versus 14%, and for the DLQI 66% versus 33%, respectively. In summary, the combined application of IPL with RF appears to represent a possible effective therapeutic option in HS, especially for Hurley I/II patients. The patients with mild to moderate HS are exactly those patients, whose disease severity does not necessarily justify treatment with anti-TNFα and hence do not have access to other approved treatment options. Additional studies with larger patient cohorts are required to confirm our findings and to gain insights into possible mechanisms of action.

P055 | Hyperspektral-imaging als neues verfahren in der wunddiagnostik

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Hintergrund: Trotz diverser Technologien, die prinzipiell in der Lage sind, die Gewebeperfusion zu visualisieren, eignen sie sich nur bedingt für den klinischen Einsatz in der täglichen Praxis und z.B. im OP. Daher war es bisher nicht möglich, den Perfusionsstatus von Problemwunden und z.B. die Effekte perfusionsfördernder Behandlungsverfahren unter klinischen Bedingungen zu überprüfen. Seit der seit kurzem verfügbaren miniaturisierten Hyperspektraltechnologie sind technisch die Voraussetzungen gegeben, dieses Problem zu lösen und Aussagen zur Perfusion schwieriger Wunden wie Transplantatwunden und der Wirkung von Therapieverfahren zu erlauben. Wir untersuchten die Eignung des Verfahrens im klinischen Routineeinsatz bei verschiedenen Wundtypen.

Methoden: Hyperspectral imaging (HSI) kombiniert digitales Imaging und Spektroskopie über 500 -1000 nm im sichtbaren und Nah-Infrarotbereich. HSI erfasst ein optisches Spektrum von jedem Bildpixel mit einer spektralen Auflösung von 5 nm und 100 Spektralkanälen. Dabei werden dreidimensionale sog. Datacubes mit 2 räumlichen und einer spektralen Dimension generiert. Auf der Basis der Spektralinformationen kalkuliert die Software über komplexe Algorhythmen die distinkten mikrozirkulatorischen Parameter mit oberflächlicher und tiefer (bis 6 mm) Sauerstoffsättigung sowie der Wasserverteilung im Gewebe. Patienten mit systemischer Sklerodermie, frischen Operationswunden, akuten und chronischen Ulkuswunden sowie Abszessen durch Problemkeime wurden mit der HSITechnologie untersucht und die Daten mit und ohne Intervention (z.B. Kaltplasmaund Infrarotbehandlung) auf klinische Wirkung und Plausibilität geprüft.

Ergebnisse: Bei allen untersuchten Erkrankungen konnten mittels HSI in Echtzeit klinisch plausible Daten gemessen und mit den klinischen Befunden korreliert werden. Bei Patienten mit Systemsklerose zeigten sich an den Händen Areale mit wechselnder Perfusion sowie Ödembildung, wobei die parametrischen Messdaten (points of interest, POI) mit den makroskopisch betroffenen Herden korrelierten. Die Perfusion konnte mittels Infrarot- und Kaltplasmabehandlung gesteigert werden, der Effekt konnte in Echtzeit am Patienten verfolgt und noch 20 minutes nach Ende der Behandlung mit dem Gerät visualisiert werden. Bei der Untersuchung der postoperativen Heilung nach Dupuytren-Operation konnten makroskopisch verdächtige Herde (V.a. Minderperfusion) erfolgreich überwacht werden. Bei der Untersuchung von sichtbaren und nicht sichtbaren Abszessen sowie iner infizierten Traumawunde konnte deren Ausdehnung einschließlich makroskopisch nicht sichtbarer kutaner Herde mit dem Verfahren sicher diagnostiziert werden (Entzündungsnachweis über Mehrperfusion mit hoher 02-Sättigung).

Fazit: Mittels HSI gelingt ohne aufwändige Einarbeitung und technischen Aufwand in Echt-Zeit die Erfassung der hämodynamisch wichtigsten Perfusionsparameter einschließlich der Sauerstoffsättigung in verschiedenen Gewebstiefen bis ca. 6 mm. Die erlaubt die Quantifizierung der Zielparameter ausgesuchter Points of Interest (POI). Erstmalig können mit dem Verfahren am Patientenbett Perfusion, Inflammation und Ödembildung größerflächiger Haut- und Wundareale sowie Infektionsherde detektiert, quantifiziert und ohne großen Aufwand rasch verlaufskontrolliert werden.

P056 | 2-Methoxyestradiol - A novel prognostic marker in malignant melanoma?

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Background: Human malignant melanoma (MM) is a life threatening and very aggressive tumor with increasing incidence and mortality rates worldwide. However, the prognostic outcome of patients is mainly based on histopathological findings and early identification of distant metastasis by staging examinations. So far, the serum markers LDH and S100B are used for prognostic assessment during later stages of disease, but failed to detect early stages of disease which is important for survival outcome of patients. Several findings suggested that 2-ME, an endogenous metabolite of 17β-estradiol, exhibits anti-tumorigenic activities. Therefore, we hypothesized that 2-ME may act as a physiological anti-tumorigenic factor which allows prognostication of MM behavior.

Objectives: The aim of this study was to examine the feasibility of using serum 2-ME as a prognostic marker for patients with MM in all stages of disease.

Methods: In this study we used a well-established ELISA to detect serum levels of 2-ME in patients with MM (Stages I-IV). We analyzed a cohort of 80 melanoma patients from the university departments of dermatology in Frankfurt and Mainz, Germany.

Results: As expected, we found in our study significant elevated levels of serum 2-ME in pregnant control patients. Unfortunately, we could not demonstrate any association between 2-ME serum levels, stages of disease, tumor thickness, LDH and S100B. Taken together, we found that 2-ME was unable to predict low- or high-grade disease.

Conclusions: We found that serum 2-ME levels failed to predict in an independent cohort of MM patients low- and high-grade disease. This highlights the need for further investigations for the role of 2-ME in cancer development and its possible use as an anti-cancer agent.

P057 | Real life treatment with omalizumab is safe and effective in patients with cholinergic urticaria

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Background: Cholinergic urticaria (CholU) is a frequent skin disorder that presents with small itchy wheals upon physical exercise. Omalizumab is effective and safe in patients with chronic spontaneous urticaria. Little is known about the effects of omalizumab in patients with CholU.

Aim: To assess the real life effectiveness of omalizumab treatment in CholU patients.

Method: We assessed 16 CholU patients for their response to omalizumab treatment and how these were linked to the dosing and duration of the treatment as well as to clinical features.

Results: 11 of 16 CholU patients (69%) reported a major or complete response, 2 patients were partial responders (13%), and 3 patients did not benefit from omalizumab therapy. 6 patients did not achieve complete disease control with the initial dose of omalizumab applied (150 mg or 300 mg/4 weeks) and were updosed. This improved clinical responses in two thirds of these patients. Omalizumab treatment was safe and well tolerated.

Discussion: Omalizumab real life treatment is effective and safe in the majority of CholU patients, but updosing may be needed and works in most patients.

P058 | Ingenol mebutate for mycosis fungoides

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The objective of the study was to assess the immunologic effects of topical ingenol mebutate treatment on mycosis fungoides skin lesions and to correlate them with the clinical efficacy of the drug. We investigated the course of three patients with early stage mycosis fungoides (MF) who applied ingenol mebutate gel once daily for three consecutive days to MF skin lesions on a three-week basis for total of 12 week. We used clinical scores and skin biopsy specimens, taken at baseline and at week six, to assess changes in disease severity. All patients showed a gradual improvement of the MF, achieving a 75% reduction in the Composite Assessment of Index Lesion Severity (CAILS) by week 12.

Limitations: The small number of patients (n = 3) limits efficacy analysis and warrants prospective placebo-controlled studies in larger patient cohorts. Topical ingenol mebutate could be beneficial for patients with mycosis fungoides.

P059 | Safety of systemic psoriasis treatments evaluated in the swiss dermatology network for targeted therapies (SDNTT)

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Introduction & Objectives: The spectrum of antipsoriatic systemic therapies is constantly changing. The short-term efficacy and safety of the various therapies have been reported in randomized and controlled clinical studies. The Swiss Psoriasis Registry SDNTT aims to gain long-term evidence of safety and effectiveness in routine care. Interim data concerning long-term safety of the first 5 years is presented.

Materials & Methods: The non-interventional Swiss Psoriasis Registry SDNTT (Swiss Dermatology Network for Targeted Therapies) observes adult patients with moderate to severe psoriasis with or without psoriatic arthritis. Patients are registered at the start of a new systemic treatment and are observed in routine care. The registry aims to gain long-term evidence of safety and effectiveness of systemic antipsoriatic drugs. Data is collected in academic outpatient clinics. The occurrence rates of AE and SAE between biologic and non-biologic treatment regimes are compared. Standardised patient rates of drug-related safety events per 100 patient years (py) of exposure classified by system organ classes of MedDRA® (SOC, Medical Dictionary for Regulatory Activities) were used for calculations.

Results: Between October 2011 and December 2016, 473 patients were included in the registry. 35% were female. The mean age was 46.7 years. 37% suffered of both psoriasis and psoriatic arthritis. 61% had nail involvement. Since the start of the registry in 2011, 264 patient years of biological and 272 years of non-biological systemic treatment were observed. Patient rates of non-serious adverse events (AE) of gastrointestinal nature were lower in the biologic compared to the non-biologic cohort (4.3 vs. 14.1/100 py, $P \leq .05$). Patient rates of non-serious infections did not differ significantly although a trend towards more frequent occurrence in the biologics cohort existed. Likewise patient rates of hepatobiliary, blood, lymphatic and reproductive tract disorders did not differ significantly, although a trend towards an increase in the non-biologic cohort was visible. Serious adverse events (SAE) including death, malignant neoplasms and others did not differ significantly between the treatment cohorts.

Conclusions: Taken together, a significant increase in gastrointestinal adverse events was observed in the non-biological treatment cohort. SAE were few in number and equally distributed between biological and non-biological treatment cohorts. We expect that higher inclusion numbers in the registry and more observation time will in the future allow further stratification to individual compounds and other parameters.

P060 | HLA-Cw6 status does not influence the therapy response in psoriasis patients treated with Secukinumab

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Introduction: A possible correlation of genetic markers and response to treatment has been proclaimed. The strongest association has been

shown between HLA-Cw6 and ustekinumab. However, no data for the potent IL-17 inhibitor, secukinumab, has been published.

Aim of study: We focused on the treatment success of HLA-Cw6 +/− psoriasis patients receiving secukinumab.

Materials & Methods: This retrospective study included 18 patients under IL-17 inhibitor treatment. Psoriasis Activity Severity Index (PASI) 75 was evaluated. Our secondary endpoints included PASI 50 and 90 as well as average PASI improvement. For statistical analysis, the unpaired t-test was used.

Results: We saw no difference between in the treatment response between HLA-Cw6 +/− patients.

Discussion: A HLA-Cw6 positive allele did not alter the treatment response in our secukinumab patients. Routine genotyping to increase the predictability of treatment success is not supported by our data.

P061 | Direct modulation of the Cutibacterium strain population as novel tool in skin microbiome research

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Recent advances in next generation sequencing technologies enabled scientists to analyze microbial communities living on the skin without the need to isolate and culture individual strains. These new technologies uncovered many associations between changes in the composition of the microbiome and resulting diseases. Nevertheless the downside of all "omics" technologies is that they yield mostly descriptive data, leaving mechanistical questions unanswered.

To unravel the pathogenic relationship between the composition of the skin microbiome and enable new therapeutic interventions, complex mixtures of heterologous skin bacteria must be established on the skin modulating the composition of its microbiome. The host's response to this intervention will subsequently allow to answer if the bacterial community is just a bystander or active player in the disease. One of these diseases is Acne vulgaris. Many recent publications showed that certain strains of Cutibacterium acnes (formerly known as Propionibacterium acnes) are associated with higher disease activity.

We isolated pure strains from healthy donors and screened them for multiple biomarkers. Four specific strains were selected. They were grown as pure cultures and applied to acne patients in a clinical study (DRKS00011297) approved by the Ethics Committee. We monitored the Cutibacterium strain level composition during 5 weeks of therapy with twice daily application in the entire facial area.

A statistically significant engraftment of the applied strains could be detected in about half of the subjects while no adverse effects were observed. We discovered significant interindividual difference regarding the engraftment of the applied bacteria. For most subjects this engraftment was stable throughout the course of the study with two exceptions. Subjects which showed positive for engraftment could be characterized by a statistically significant increase of the relative abundance of *C. acnes* in relation to other species. Subjects which were negative for engraftment had a higher relative abundance of *C. acnes* at the start of the study and returned to this number after a temporary increase.

Our study demonstrates that heterologous strains of bacteria can be established in a skin microbiome. This enables researchers to interrogate the role of different skin commensals in diseases for which microbiota may contribute to the pathogenesis.

P062 (OP04/03) | Therapeutic inhibition of IL-17 leads to clinical remission of lichen planus

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LP is a common chronic inflammatory disorder of skin and mucous membranes whose immune pathogenesis has been linked to T cell-mediated cytotoxicity against epidermal keratinocytes. Recently, we identified autoreactive Th1 and Th17 responses in a cohort of LP patients which recognized bullous pemphigoid antigen 180 (BP180) and desmoglein 3 (Dsg3), well-known autoantigens of the skin. Of note, BP180-reactive peripheral Th17 cells were significantly increased in LP patients with mucosal and skin lesions. We here studied the clinical efficacy of secukinumab, a monoclonal antibody against IL-17a, in three patients with acute and chronic recalcitrant muco-cutaneous LP and the efficacy of ustekinumab, a monoclonal antibody which selectively binds the subunit p40, a protein shared by both IL-12 and IL-23, in a patient with a chronic recalcitrant mucous lichen planus. Secukinumab was applied for 13 weeks and the patients were monitored clinically by the Autoimmune Bullous Skin Intensity Score (ABSIS) and immunologically (analysis of lesional cutaneous T cell subsets and peripheral BP180- and Dsg3-specific T cells by immunohistochemistry and Elispot analysis before, during and after secukinumab treatment. After 13 weeks of therapy, all the three LP patients (P1-3) showed a remarkable clinical resolution of the skin and mucosal lesions with a clear decrease of the ABSIS scores (ABSIS I: P1: 5 to 0, P2: 7 to 2, P3: 3.5 to 1; ABSIS II: P1: 45 to 0, P2: 21 to 0, P3: 11.5 to 0). This was accompanied by a strong reduction of the inflammatory skin infiltrate and a relative decrease of lesional T cells (percentage of $CD3^+$ cells of all infiltrating cells before therapy: 52.8%±15, after therapy: 39.1%±5.5). Although BP180- and Dsg3-specific Th1 and Th17 cells were detectable throughout the observation period, we could not detect a decrease of autoreactive Th17 cells upon treatment with secukinumab. In addition, secukinumab treatment did not affect distinct $CD4^+$ and $CD8^+$ T cell subsets suggesting that the observed therapeutic efficacy was related to the neutralization of soluble IL-17. Of note, ustekinumab treatment also showed remarkable clinical efficacy in a patient with oral lichen planus which was reflected by the ABSIS scores (ABSIS I 7 to 2; ABSIS II 34 to 7.5). Upon secukinumab treatment, plasma levels of IL-17A and RANTES were decreased MIP-α

and IL-10 plasma concentrations were increased. These findings show for the first time that mucosal and cutaneous LP rapidly responds to therapeutic inhibition of IL-17A and of IL-23 and strongly support the concept that Th17 cells are critically involved in the immune pathogenesis of LP.

P063 | Think big and scale down: development of a multi-organ-on-the-chip-model for analysis of immune cell - skin interactions

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To date the interaction between skin and the immune system in the context of chronic inflammatory skin diseases is poorly understood, complicating the development of targeted therapeutic strategies. To address this need, we aimed to establish a multi-organ-on-the-chip system in which human skin and immune cells can be cultured together in a dynamic miniaturized human chip culture. This system closely resembles the activity of multiple human organs in their true physiological context at the smallest possible biological scale. Thereby it provides the possibility to simulate the crosstalk between skin and immune cells as close to the human in vivo situation as possible. The suitability of multi-organ chips combining skin and immune cells was tested exemplary by establishing a skin allograft on-the-chip model. Our results show, that integration of healthy donor's skin and allogeneic immune cells provokes a characteristic cytokine pattern of allograft rejection. This was accompanied by histological signs of tissue destruction. In summary, we conclude that our model might also be suitable to investigate underlying pathogenetic mechanisms in chronic inflammatory skin diseases such as psoriasis, atopic dermatitis or acne inversa. Consequently, this system might enable the development and testing of novel therapeutic approaches with the advantage of reducing animal studies in accordance with the 3R principle.

DERMATO-ENDOCRINOLOGY

P064 | The antimicrobial peptide koebnerisin (S100A15) regulates rosacea key molecules and is controlled by doxycycline

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Rosacea is a common inflammatory skin disease. An aberrant innate immune response to external stimuli may play an essential role in the pathogenesis of rosacea. The antimicrobial peptide koebnerisin (S100A15) is an innate immune active player with diverse inflammation-regulating functions. Here, we investigated the expression of koebnerisin in rosacea skin lesions by quantitative RT-PCR and immunofluorescent analysis and assessed the function of koebnerisin in rosacearelevant skin cells. We could show that koebnerisin is overexpressed in rosacea skin lesion and that koebnerisin may exert anti-inflammatory functions in keratinocytes by suppression of vascular kallikrein 5 (KLK-5), matrix metalloproteinase-9 (MMP-9), Toll-like receptor 2 (TLR-2), and cathelicidin (LL-37). Subantimicrobial doses of doxycycline are an established treatment option for rosacea. Data revealed that koebnerisin and its rosacea key targets are regulated by doxycycline in keratinocytes and dermal fibroblasts. In conclusion, koebnerisin emerges as a novel player in the pathogenesis of rosacea where balancing the innate immune response might be promising for future therapeutic interventions.

P065 | The secretome of skin cancer cells influences the transcriptome of keratinocytes

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The secretome of cells has been defined as a class of proteins constituted by the extracellular matrix and by secreted proteins, such as cytokines, chemokines, growth factors and proteases. 11% of human genes, corresponding to about 3000 genes, encode proteins that will be secreted. In the skin and most other tissues this fraction ranges between 10 and 20%, while in organs like the pancreas and the salivary glands it is much higher. 15% of all FDA-approved drugs target secreted proteins, and that makes the secretome an attractive reservoir of druggable proteins. The secretome of cancer cells has recently been the object of many oncoproteomics studies, as it represents a pool of putative tumor biomarkers and pharmaceutical targets. Nevertheless, it is still not known how or if these elements are affecting the surrounding healthy cells during tumor development. There are two main types of skin cancer: non-melanoma skin cancer (NMSC) and melanoma skin cancer. NMSC arises from keratinocytes, and, based on the types of cells it develops from, it is further divided into basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). BCC is the most common type of skin cancer, as it accounts for 80% of all cases, while SCC accounts for 16%. The remaining 4% is caused by melanoma skin cancer, the most aggressive and dangerous type of skin cancer. Melanoma skin cancer develops from melanocytes, pigment-producing cells in the epidermis and, contrarily to NMSC, it often metastasizes across the body. To identify the secretome of human keratinocytes

and the three skin cancer types, we employed the following approach. We cultured healthy human keratinocytes (HaCaT), A431 (SCC), TE 354.T (BCC) and A375 (melanoma) cancer cells in serum-free medium for 24 hours and collected the culture medium (CM) containing the secretome and the cell pellet. Both fractions were analyzed by tandem mass spectrometry (LC-MS/MS). After quantification, we determined the proteins enriched in the CM fraction vs. the pellet fraction and then carried out bioinformatics analysis. We were able to characterize 620 enriched proteins in the CM of HaCaT cells, 603 in the CM of A431, and 82 in the CM of A375. In a second experiment, we incubated HaCaT keratinocytes for 24 hours with the CM obtained from A431 cancer cells and analyzed the transcriptome by next-generation sequencing. We identified more than 1000 significantly up- and down-regulated genes in the transcriptome of HaCaT cells incubated with the CM of A431 cells compared to control HaCaT cells. More specifically, tumor suppressor genes like TP53 were down-regulated in keratinocytes by the secretome of A431, while protooncogenes like MYC were up-regulated. In conclusion, the results demonstrate that the secretome of cancer cells exerts an important impact on the transcriptome of healthy cells.

P066 | The alpha7 nicotinic acetylcholine receptor - a crucial modulator of cutaneous and extracutaneous fibrosis?

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Our previous results demonstrated that the alpha7 nicotinic acetylcholine receptor (alpha7nAchR) agonist tropisetron and the full alpha7nAchR agonist AR-R17779 both inhibited TGF-beta-induced collagen synthesis in human dermal fibroblasts (HDFs). Moreover our in vivo data disclosed that tropisetron can not only reduce but also prevent experimentally-induced skin fibrosis in the bleomycin (BLM) mouse model of scleroderma. Mechanistically, neither the SMAD nor the cAMP or MAPK signaling pathway was involved in the antifibrotic effect of tropisetron. To further investigate the molecular mechanism of the anti collagenic effect of tropisetron we focused on the activator protein (AP)-1 signaling pathway, since this factor was shown to play a role in collagen turnover. Our results show that stimulation of HDFs with TGF-beta induces the expression levels of the AP-1 members c-Jun, c-Fos and ATF3. However, coincubation with tropisetron does not modulate the TGFbeta- mediated upregulation of mRNA and protein expression of these factors. Therefore, we tested 2 micro (mi)RNAs which are implicated in regulation of fibrosis. The profibrotic miRNA-21 was induced after TGF-beta treatment in our cells whereas coincubation of TGF-beta with two specific alpha7nAchR agonists (AR-R17779 and PHA 543613) abrogated the TGF-beta-induced upregulation of miRNA-21 mRNA expression. In accordance, the expression of the antifibrotic miRNA-29 was significantly upregulated by these two agonists in our cells in comparison to TGFbeta alone at RNA level. These results indicate that the anti-collagenic effect of the alpha7nAchR could be mediated via activation of specific miRNAs. To explore the further role of the alpha7nAchR in fibrosis we will extend our investigations beyond the skin to extracutaneous fibrosis models. Therefore experiments with BLM induced lung fibrosis and CCl4-induced liver fibrosis as well as fibrosis studies with alpha7nAchR-deficient animals are already in preparation in our laboratory.

P067 | International Hidradenitis Suppurativa Severity Score System (IHS4): a novel dynamic scoring system to assess HS severity

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The exponentially increasing interest in hidradenitis suppurativa/acne inversa (HS) highlights the need for validated, easy to use disease severity assessment tools that can be used both in clinical trials and daily clinical practice and which accurately classify severity and guide the therapeutic strategy. Therefore, a Delphi voting procedure was conducted among the members of the European Hidradenitis Suppurativa Foundation (EHSF) to achieve consensus towards an initial HS Severity Score System (HS4). Strengths and weaknesses of HS4 were examined by a multicenter prospective study. Multivariate logistic regression, discriminant analysis and receiver operating characteristic curves as well as examination for correlation (Spearman's rho) and agreement (Cohen's kappa) with existing scores were engaged to recognize the variables for a new International HS4 (IHS4) that was established by a second Delphi round. Consensus HS4 was based on the number of skin lesions, the number of skin areas involved and the Dermatology Life Quality Index (DLQI), and was evaluated by a sample of 236 patients from 11 centers. Subsequently, a multivariate regression model calculated adjusted odds ratios for several clinical signs. Nodules, abscesses and draining tunnels resulted as the scoring variables. Three candidate scores were presented to the second Delphi round. The resulting IHS4 score is arrived at by the number of nodules (multiplied by 1) plus the number of abscesses (multiplied by 2) plus the number of draining tunnels (multiplied by 4).

A total score of 3 or less signifies mild, 4-10 signifies moderate and 11 or higher signifies severe disease. Cohen's kappa was fair (= 0.317) compared with Hurley classification, and moderate (= 0.493) compared with Expert Opinion. Correlation was good (rho > 0.6) with Hurley classification, Expert Opinion, Physician's Global Assessment and Modified Sartorius score, and moderate for DLQI (rho = 0.356). In conclusion, the novel IHS4 is a systematically constructed, validated and simple tool to dynamically assess HS severity and can be used both in daily practice and the clinical trials setting.

P068 | Digital assessment and cinematography of acne lesions

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A precise and reliable assessment of acne severity is unarguably the most essential clinical characteristic when it comes to monitoring and choosing optimal treatment in the daily practice. Since the early 1960s different severity assessment systems have been described in the literature. The two commonly used concepts are global gradings and lesion counting. Both, have been controversially discussed as the more effective and objective outcome measurement tool. Regardless of these discussions, both suffer from the underlying issues in the form of rater subjectivity. Advanced methods to assess the severity of acne vulgaris such as photography, fluorescence photography, polarized light photography, video microscopy, multispectral imaging have been developed and adapted to gain more objective measurements. However, they have not yet been established due limiting factors like high cost, complex and sophisticated apparatuses, and a sometimes time consuming imaging process. Newly developed technologies have been identified, such as the Software-Assisted Acne Auto-Classification and the Acne Cinematography, which could solve the discrepancies that arise from inter- and intra-rater subjectivity.

P069 | Medical infrared thermography (IRT): a new technique to visualize skin inflammation and to provide objective evidence of therapeutic effectiveness

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Infrared thermography (IRT), a non-invasive and radiation-free imaging method, has been used in medicine for the detection of tissue abnormalities beneath the skin, e.g. of the joints. Correlations between joint inflammation and IRT parameters, including thermographic index, heat distribution index, mean and maximum temperature have been reported. The technique has currently been transferred from large instruments to small handheld units, which allow a wider clinical use. Changes in thermal patterns of skin temperature have also been demonstrated in the area of joint inflammation. Therefore, the technique could be applied to detect changes of skin temperature and their magnitude in inflammatory skin diseases. Moreover, it can be employed to objectively detect the therapeutic success of anti-inflammatory treatment. At last, the real-time IRT modus can be introduced to assist the surgical treatment of inflammatory skin diseases, such as hidradenitis suppurativa/acne inversa. The technique facilitates a pre-operative mapping of the surgical target up to disease-free margins and can be used additionally to optimize the effectiveness of surgical excision, by real-time visualization of the inflammatory tissue.

P070 | Selective role of epithelial skin cell RIS-1/psoriasin expression in innate immunity and in inflammatory skin diseases

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RIS-1/psoriasin/S100A7 is an epithelial antimicrobial peptide, whose expression is upregulated in inflammatory skin diseases and induced by retinoids. Its molecular expression was investigated in skin cell cultures and in skin specimens in order to better understand its role in inflammatory procedures of the pilosebaceous unit. rtPCR and northern blotting of RIS-1/psoriasin and the retinoid-metabolizing genes CYP26AI and CRABP-II were performed in cells cultures (keratinocytes, sebocytes, fibroblasts, endothelial cells, melanocytes, lymphocytes and prostate cells; native and treated with retinoids) and in situ hybridization in normal and inflamed skin (acne, psoriasis). RIS-1/psoriasin is expressed in keratinocytes and fibroblasts in vitro and in keratinocytes of the stratum granulosum in vivo. Retinoids in vitro and inflammatory conditions in vivo increase the levels of RIS-1/psoriasin in keratinocytes (both), sebocytes (inflammation only) and fibroblasts (retinoids). Sebocytes and fibroblasts are the metabolically most active skin cells, since they can upregulate the expression of CRABP-II and CYP26AI, genes responsible for retinoid metabolism. Inflammation modifies the compartmentation of RIS-1/psoriasin in sebaceous glands and the follicular root sheaths. These data indicate that anti-inflammatory treatment targeting the epithelial compartments of the skin, including such with antibacterial peptides, may be promising for the treatment of inflammatory skin diseases.

P071 | Sebocyte apoptosis induced by free fatty acids is dependent on caspase activity

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Production and release of sebum on skin surface ensures skin suppleness and contributes to its intact barrier function. With increasing age this function is disturbed leading to drier skin and high susceptibility to microbial infections. Knowledge regarding the regulation of lipogenesis in sebocytes and subsequent release of sebum components through apoptosis still remains sparse. In this study, we focused on the apoptotic response of the human sebaceous gland cell line SZ95 to four biologically relevant fatty acids, arachidonic acid, linoleic acid, palmitic acid and palmitoleic acid. We report for the first time that high basal sebocyte apoptotic rate is strongly suppressed by the pan-caspase inhibitor QVD-OpH, thus underlining the dependency on proapoptotic caspase cascades. Furthermore, all four fatty acids induced the accumulation of cytoplasmic lipid droplets with subsequent apoptosis and cell death, with arachidonic acid causing the most potent effect. Fatty acid-induced apoptosis could be markedly inhibited by pre-incubation with the pan-caspase inhibitor but lipid droplets accumulated further. While cell viability after incubation with linoleic acid, palmitic acid or palmitoleic acid and QVD-OpH was comparable to controls, arachidonic acid caused a significant decrease in cell viability in spite of pan-caspase inhibitor treatment. Real-time cell analysis (RTCA) also showed a significant cell density decrease of arachidonic acid-treated SZ95 sebocytes even in combination with QVD-OpH, compared to the other three treatments and untreated SZ95 sebocytes. These findings indicate that different fatty acid stimuli cause different responses of the sebocytes with regard to lipogenesis and apoptosis, but all using caspase pathways. This can contribute to a better understanding of the mechanisms underlying lipogenesis and cell death induction in human sebocytes.

P072 | IGF-I and oxidative stress regulate expression of fzd7 and downstreams in old epidermal stem cells

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Disturbed microenvironmental conditions e.g. reduced hormone or growth factor release or increased local oxidative stress influence epidermal skin homeostasis at high age. In previous data, we have demonstrated that frizzled 7 (fzd7) a receptor involved in canonical and non-canonical Wnt signaling pathway is disturbed in aged epidermis affecting stem cell activity and the proliferation potential of epidermal stem cells. In order to assess the factors contributing to this dysfunction we investigated the effects of insulin-like growth factor I (IGF-I) - a growth factor known to decline with age in both genders- and of rotenone - a complex I inhibitor of oxidative phosphorylation. After treatment of old murine epidermal stem cells with IGF-I in a time and dose-dependent manner, protein and mRNA expression of fzd7 and of downstreams involved in the canonical (e.g. β-catenin) and the non-canonical Wnt pathway (e.g. cdc42) showed to be significantly regulated by means of Western blotting and real-time PCR, accordingly. Particularly, high levels of IGF-I significantly stimulated protein expression levels of fzd7 and active β-catenin ($P<.01$ and $P<.001$, respectively), while active cdc42 showed to be significantly downregulated after 12 hours treatment ($P<.01$). Furthermore, treatment of epidermal stem cells with 0, 10 μM and 100 μM rotenone showed a significant induction of fzd7 and active β-catenin at 100 μM ($P<.01$) indicating that only high levels of oxidative stress induce Wnt signaling activation. Taken together, our data illustrate that reduced IGFI levels with age mostly due to fibroblast senescence enhance activation of the noncanonical Wnt pathway in epidermal stem cells. In addition, high levels of oxidative stress induce canonical Wnt signaling activation in epidermal stem cells as observed in regeneration and cancerogenesis.

P073 | Impact of the melanocortin tripeptide derivative KdPT on wound healing, NFB signalling and barrier function proteins in human keratinocytes in vitro

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KdPT is a melanocortin tripeptide derivative that was shown to have antiinflammatory effects in vitro (Mastrofrancesco et el., 2010) as well as in murine models of experimentally induced colitis (Bettenworth et al., 2012). In the latter models KdPT also modulated the localization of various tight junction (TJ) proteins in the colonic epithelium which are known to control barrier function. Importantly, KdPT does not bind to the melanocortin 1 receptor and therefore has no pigment-inducing effect. We wondered if KdPT affects cutaneous wound healing and alters expression of TJ proteins implicated in the barrier function of the epidermis. KdPT per se did not have any modulatory effects on the viability, metabolic effect and proliferation of human epidermal keratinocytes. We further found that KdPT at 10^{-6} M and 10^{-8} M attenuated the effect of tumour necrosis factor (TNF) on mechanically induced wounds in scratch assays. KdPT alone, in contrast, had no detectable effect on wound closure in this model. To elucidate the mechanism behind this action of KdPT we analysed various components of canonical NF-B signalling. TNF under the given experimental

conditions did not induce oxidative stress. KdPT likewise did not affect the amount of intracellular hydrogen peroxide detectable in human epidermal keratinocytes. In accordance with this expression of IBalpha was neither affected by TNF nor KdPT. Further, expression and subcellular localization of p65 did not change in human keratinocytes treated with any of these agents. Finally, expression and immunolocalization of claudin-1, -3, occluding and zeocin was examined by real-time PCR and Western immunoblotting in cells treated with TNF and KdPT under low and high levels of extracellular calcium. In contrast to cells exposed to high extracellular calcium no consistent changes in the expression or localization of the above TJ protein could be detected. In sum, our results highlight a wound healing-promoting effect of KdPT in human keratinocytes in presence of TNF. This observation is consistent with our findings of KdPT as a modulator of wound healing under high-glucose-induced stress (Gkogkolou et al., in revision).

DERMATOPATHOLOGY

P074 | Direct pathogenicity of anti-Desmoglein autoantibodies cloned from the IgA1 repertoire of a patient with IgA pemphigus

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IgA pemphigus is an autoimmune blistering skin disease characterized by detection of tissue-bound and circulating IgA, but not IgG, autoantibodies (autoAbs) against cell surface proteins of epidermal keratinocytes. Patients typically present with vesiculopustular skin eruptions, intraepidermal acantholysis, and prominent neutrophilic infiltrates, suggesting a critical role of neutrophils in disease induction. To facilitate dissection of pathomechanisms involved, we applied antibody phage display to clone anti-Desmoglein (Dsg) 3 monoclonal antibodies (mAbs) from a patient with clinically active IgA-Pemphigus vulgaris (IgA-PV), as confirmed by positive skin biopsy immunofluorescence (IF) and exclusive serum anti-Dsg3 reactivity; no Dsg1 or Desmocollin reactivity was detected. We obtained 3 Dsg3-specific IgA mAbs, in form of single-chain variable fragments (scFv), genetically derived from 2 B-cell clones displaying VH3-23 gene usage. Our scFv demonstrated binding to Dsg3 but not Dsg1 as shown in ELISA and IF of transfected HEK-cells. To validate our mAbs, we performed inhibition ELISA studies using sera from the same patient, from unrelated active IgA-pemphigus patients (with similar serum reactivities), and from active IgG-PV patients with Dsg3-IgG abs. All these sera led to decreased binding of our scFvs indicating that they bind to similar, or identical Dsg3 epitopes also bound by polyclonal serum abs. This finding illustrates the biological validity of our isolated mAbs, further confirmed by cell sheet dissociation of cultured human keratinocytes in a dose-dependent manner. Similarly, our mAbs displayed pathogenic activity in human skin organ culture assays (under elimination of Dsg1 by exfoliative toxin A), resulting in blister formation. These findings indicate that anti-Dsg3- IgA autoAbs are necessary but not sufficient for disease induction in our patient. Therefore, we reasoned that FcáR-bearing cells contribute to disease induction and are currently producing recombinant full IgA1-mAbs from above scFvs to study cellular mechanisms of tissue pathology involved in IgA-mediated autoimmune skin disease in vitro. Shown cross-reactivity of our Dsg3-IgA mAbs with murine keratinocytes offers an excellent platform to extend these studies to in vivo models, allowing for development and testing of targeted therapies against this treatment resistant disorder.

P075 (OP01/01) | Rationale for anti-IL-17A treatment in bullous pemphigoid

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IL-17A has been identified as key regulatory molecule in several autoimmune and chronic inflammatory diseases, resulting in the successful use of anti-IL-17 therapy, e.g. in rheumatoid arthritis, multiple sclerosis, and psoriasis. Bullous pemphigoid (BP) is the most frequent autoimmune blistering disease with a high need for more specific, effective and safe treatment options. Previous studies suggested a pathogenic role of IL-17 in BP while the cellular source and functional relevance of IL-17 in BP have not yet been fully clarified. Here, we found elevated numbers of IL-17A$^+$CD4$^+$ lymphocytes in the peripheral blood of BP patients (n=15) and identified both neutrophils and CD4$^+$ cells as major source of IL-17A in early BP skin lesions (n=7). IL17A and related genes such as CXCL1 and IL6 were found to be upregulated in BP skin (n=15) and appeared to have a central role in the local inflammation by connectivity analysis. The functional role of IL-17A in BP was suggested by the reduced reactive oxygen species (ROS) release of BP180- anti-BP180 IgG immune complex-stimulated neutrophils after treatment with anti- IL-17A IgG and the inhibition of dermal-epidermal separation in cryosections of human skin incubated with anti-BP180 IgG and subsequently with anti-IL-17A IgG treated leukocytes. In the experimental mouse model of BP, (i) a close correlation of serum IL-17A levels and disease activity was observed, (ii) IL-17A$^{-/-}$ and IL-17A/F$^{-/-}$ mice showed significantly less skin lesions compared to wild-type mice (P<.0001 and P=.01) with higher disease activity in IL-17A/F$^{-/-}$ compared to IL-17A$^{-/-}$ animals (P<.001), and (iii) treatment of anti-BP180 IgG-injected wild type mice with a neutralizing anti-IL-17A antibody resulted in significantly less skin lesions (P=.02).

Based on these data, a BP patient with extensive disease received adjuvant treatment with secukinumab, an IL17A blocking antibody, and long term complete remission of 11 months was induced, along with reduced IL-17A$^+$CD4$^+$ peripheral blood cells and normalization of anti-BP180 IgG serum levels without adverse events. Our data suggest a functional role of IL-17A in BP pathophysiology and point to IL-17A inhibition as a potential treatment option for this neglected disease.

P076 | STAT3 Hyper-IgE Syndrome: A way to understand the role of Staphylococcus aureus in atopic dermatitis

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Background: The bacterium Staphylococcus aureus has an important role in the pathogenesis of atopic dermatitis (AD), but it is unclear in detail whether the occurrences of *S. aureus* infections are causative or rather an epiphenomenon of chronic eczema. Patients with autosomal dominant STAT3 hyper-IgE syndrome (STAT3-HIES) show analogies to AD patients such as recurring cutaneous *S. aureus* infections and an imbalance between Th17 and Th2 immunity next to chronic eczematic lesions.

Objective: To investigate the role of *S. aureus* in chronic eczema on primary keratinocytes under Th2 and Th17 conditions.

Methods: Influence of *S. aureus* components such as Staphylococcal Enterotoxin B (SEB) under Th2 (IL-4 and IL-13) and Th17 (IL-17 and IL-22) conditions on primary keratinocytes has been investigated by using CCL2, IL-1β and IL-8 Enzyme-Linked- Immunosorbent Assay (ELISA).

Results: We confirmed published effects of SEB alone and investigate its effect in combination with the Th2 and Th17 cytokines on the secretion of the inflammatory peptides (IL-1β and CCL2) and the neutrophil recruitment peptide IL-8 in keratinocytes. We confirmed the CCL2-inducing action of the Th2 and Th17 cytokines in keratinocytes. Preliminary results showed that the IL-1β-inducing action of SEB can be overcome by the use of Th2 cytokines. Furthermore, SEB alone decreases the secretion of IL-8 in keratinocytes. IL-22 enhances SEB effects on IL-8 in keratinocytes but IL-17 reduces this inhibitory effects.

Conclusion: Our results show that the influence of SEB on the secretion of the inflammatory peptides and the neutrophil recruitment peptide IL-8 in keratinocytes is dependent on cytokine conditions. With this model, we are able to assess various components of *S. aureus* and its effect on chronic eczema by comparing keratinocytes of AD and STAT3-HIES patients with the aim to understand the role of *S. aureus* in the pathogenesis of chronic eczema.

P077 (OP01/04) | A novel urticarial and Factor XII mutation-related autoinflammatory syndrome

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Background: Early onset cold-induced urticarial rash combined with various signs and symptoms of systemic autoinflammation are linked to mutations in the NLRP3, NLRP12 and NLRC4 gene. However, in a number of familial cases, genetic tests for these mutations are negative, suggesting unknown genetic variants. Here, we describe the discovery and characterization of a novel familial cold-induced urticarial autoinflammatory syndrome in a family with a novel mutation in the human coagulation Factor XII gene.

Methods: We identified a four-generation family presenting with cold-induced urticarial rash, arthralgia, fever and malaise associated with an autosomal-dominant inheritance pattern. Genetic analysis included exome sequencing and targeted Sanger sequencing. Also, we assessed plasma levels of inflammation markers and hemostasis parameters and performed immunoblotting, stimulation assays of mononuclear cells and generated recombinant genetic constructs.

Results: Whole exome sequencing of three family members across three generations identified a rare deleterious variant in the F12 gene (T859A, resulting in W287R) that was subsequently confirmed by Sanger sequencing to segregate with all affected family members. Functional studies revealed spontaneous FXII activation in the patients' plasma and distinct 50 kDa bands in the FXII immunoblot. These were confirmed by generation of synthetic W287R constructs suggesting the introduction of a new cleavage site close to a kringle domain. C1 inhibitor concentration and activity as well as hemostasis parameters appeared normal, but strong activation of the contact system was demonstrated by reduced plasma prekallikrein (PPK) activity and profound cleavage of high molecular weight kininogen (cHMWK). Also, blood levels of the inflammation markers C-reactive protein (CRP) and S100A12 were elevated. Treatment of affected family members with the IL-1 receptor antagonist anakinra and C1 inhibitor concentrate resulted in reduced disease activity. Stimulation assays with patient plasma or the synthetic construct showed upregulation of IL-1β by mononuclear cells, which was also reducible by anakinra.

Conclusions: Taken together, our findings identify a novel autoinflammatory syndrome characterized by a genetic substitution in the FXII gene resulting in FXII activation, activation of the contact system, IL-1β-mediated inflammation.

P078 | Nummular eczema is a distinct clinical entity with overlapping features of both, psoriasis and atopic eczema

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Background: Nummular eczema (NE) is a chronic inflammatory skin disease that is characterized by multiple, pruritic, discoid-shaped eczematous lesions. However, eczema comprises a heterogeneous group of diseases in terms of clinical, histological, and molecular hallmarks with many overlapping phenotypes evident. While diagnosis is made primarily clinically in correlation with histological findings, the etiological cause and exact pathophysiology of NE are unknown.

Objectives: We sought to better understand the disease characteristics of NE and compare it to Atopic eczema (AE) and psoriasis.

Methods: A total of 31 patients with NE, 40 patients with AE and 65 patients with psoriasis confirmed by clinical and histological evaluations were included in this study. We compared clinical, histopathological and immunohistochemical patterns between these groups.

Results: NE patients in our cohort were older with a mean age of 61.2 compared to AE (39.8) and psoriasis (46.3). Disease severity was overall similar including mostly moderate and severe affected patients with a mean PGA in NE of 3.2, a mean PASI in psoriasis of 11.4 and a mean SCORAD of 42.6 in AE. Consistently, all three groups had a likewise, great reduction in quality of live, with a DLQI in NE of 11, in AE of 12.3 and in psoriasis of 10.3.

The atopic characteristics, serum IgE levels and blood eosinophils were highly significantly elevated in AE (median total IgE: 627 kU/L; eosinophilia: 71%) compared to NE (105 kU/L; 32%), while there was no significant difference between NE and psoriasis (67.4 kU/L; 19%), although both diseases presented higher IgE levels compared to healthy controls (27.9 kU/L). Moreover, colonization with Staphylococcus aureus (SA) on lesional skin was most pronounced in AE (85%). However, NE (52%) was significantly more often colonized by SA than psoriasis (17%).

Histologically, lesional epidermis showed greater acanthosis in NE (322 μm) than in AE (230 μm), but was less pronounced compared to psoriasis (484 μm). Significant intralesional neutrophilic infiltration was more often present in NE (52%) compared to AE (8%), while there was no difference considering intralesional eosinophils between AE and NE.

Consistent with the clinical and histological data, immunohistochemistry revealed a higher expression of neutrophil elastase (14 vs. 2 cells/HPF) and Ki67 (188 vs. 129 cells/HPF) and a lower expression of the Fcε-receptor (106 vs. 52 cells/HPF) in NE compared to AE.

Conclusion: Clinical and histological evidence suggest that NE is a distinct clinical entity rather than a version of AE with overlapping features of both AE and psoriasis.

P079 | T cell receptor repertoire analysis in skin lesions of the mouse model EBA revealed that the skin immigrating TCR β clonotypes change over time

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Autoimmune bullous disorders are severe skin diseases that are characterized by lesions, blisters and erosions either of the skin or mucous membranes. The development of lesions is associated with the presence of IgG autoantibodies, binding to distinct extracellular structures of the epidermis, the basal lamina, and anchoring fibrils of the dermis. The role of autoreactive B cells for pathogenesis is clear established, however, the contribution of autoreactive T cells is still poor defined. On the one hand, autoreactive T cells are required for their interaction with cognate B cells to induce IgG class switch and somatic hypermutation. On the other hand, T cells immigrate into skin lesions and secrete cytokines, which could drive the course of the disease. Little is known about the clonality of T cells enriched in skin lesions and a broad characterization of the T-cell receptor (TCR) repertoire in lesional skin compared to non-lesional skin is lacking. To find out, the skin blistering disease Epidermolysis bullosa acquisita (EBA) was induced in SJL mice by injection of the autoantigen collagen type 7 (mCol7). By using high throughput complementarity-determining region 3 (CDR3) TCRβ sequencing, the TCR β repertoire of lesional EBA skin and injured lesional skin from healthy control mice, induced by mechanical irritation, was identified. We examined the TCR β repertoire diversity and characterized the top increased clones shared among the mice, and quantified the frequencies of their TCR Vβ/Jβ segment usage.

Our data show that the frequency of TCR β clonotypes shared between the mice increases transiently within the first 4 weeks after disease induction and declines to control level within 7 weeks. Additionally, analyzing the CDR3 amino acid length revealed a shorter TCR β repertoire 2 weeks after disease induction and a uniform length among later time points and control. PCA analysis of the V/J segment usage of skin infiltrating T cells revealed a clear separation of the 4-7 week time points from early time points, mock immunized and the control group. This separation indicates that the early skin immigrating TCR β clonotypes differ from those that accumulate at later time points. We conclude that a skin injury induces a random immigration of TCR β clonotypes initially that gets replaced from new TCR β clonotypes during establishment of chronic lesions. It is reasonable to assume that especially autoreactive TCR β clonotypes that recognize their antigen in the skin accumulate over time. However, unexpectedly, the number of TCR β clonotypes in skin lesions shared among the mice does not increase, which might be due to the extremely high diversity of T cell clonotypes. In future studies it will be interesting to investigate whether identical TCR β clonotypes will emerge in distinct lesions of one mouse.

P080 | Deciphering the difference between frontal fibrosing alopecia (FFA) and lichen planopilaris (LPP): Macrophages hold the key?

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Frontal fibrosing alopecia (FFA) and Lichen planopilaris are inflammatory hair discorders leading to hair follicle (HF) destruction and scarring. Traditionally FFA has been regarded as a variant of lichen planopilaris (LPP) based on histological features, however, distinct phenotypic differences, demographics and epidemiology argue against this distinction. So far clinicians and scientists have failed to systematically describe the differences between each pathology. One avenue for investigation is the macrophage population. As macrophages are known scavengers of the immune system having a role in programmed organ deletion and also are implicated in human hair cycle control we chose to investigate their potential role in the pathogenesis of LPP and FFA. Therefore in this sturdy we have utilized quantitative immunohistomorphometry of CD68 staining to investigate the function of macrophages in LPP and FFA pathogenesis. Results showed that CD68 was prominently expressed in both LPP and FFA compared with controls with staining being prominent in partially destroyed HF, suggested by the visible disruption of the HF epithelium and conspicuous foreign-body giant cells. Comparing LPP to FFA, there was a significantly increased number of $CD68^+$ cells in LPP HFs compared with both FFA and control samples suggesting they may have a different role in LPP when compared with FFA. This was found in both in the peri-follicular and interfollicular mesenchymal spaces. These pilot results suggest a role for macrophages in LPP and FFA. That the macrophage population was more prominent in LPP suggests they may have a more substantial role in the pathogenesis of LPP than FFA. Next it is essential that the polarization of macrophages is characterized to investigate what role these immune cells have in pathogenesis, i.e. a scavenger role or whether they act in as secretory cells producing cytokines such and IFN that promote the loss of immune privilege and stem cell destruction. Our preliminary study provides the first evidence suggesting a subtle yet important difference in the pathogenesis of LPP and FFA. By understanding the differences between each condition we can aim to devise better targeted treatment strategies to prevent and restore hair loss leading to better clinical outcomes.

P081 (OP04/05) | Functional specialization of inflammatory dermal monocytes in psoriasis

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Psoriasis is a common chronic inflammatory skin disease. The clinical picture results from hyperproliferative epidermal keratinocytes, a dense leukocyte infiltrate in the skin and dermal hypervascularization. We reported on the role of 6 sulfo LacNAc (slan) dendritic cells serving as TNF-α producing inflammatory dermal dendritic cells (TIP-DCs) by driving Th17/Th1 responses in psoriasis. In addition to psoriasis, these proinflammatory slan+ cells have also been described in inflammatory bowel disease, multiple sclerosis as well as in rheumatoid arthritis. Interestingly, as revealed recently, slan+ cells share a transcriptomic signature with monocytic cells and should - in reference to an ontogeny driven nomenclature - now be called slan+ monocytes (slanMo). The highly selective expression of slan among a specific subset of human proinflammatory mononuclear phagocytes (MP) allows the development of therapeutic in vivo targeting strategies for the depletion of these cells. Accordingly in a first step, we are aiming to develop an efficient strategy to target slanMo, and their cellular depletion. In addition, this approach requires an in vivo model that allows studying the proinflammatory role of slanMo in detail.

For targeting slanMo, we generated a biotinylated single chain fragment variable (scFv) from the slanMo specific IgM mAb clone DD2. These biotinylated-scFv trimerize with streptavidin-PE, which bind to the slan carbohydrate moiety on the PSGL-1 and identify slanMo with a similar efficiency as the original DD2 mAb. Furthermore, these biotin-scFv can be complexed via streptavidin to a trivalent, saporin-based immunotoxin. Upon scFv-directed antigen targeting and internalization, the plant-derived saporin toxin is released into the cytoplasm and causes cell death within 72 hours due to ribosome inactivation. This ablation system is in principle diversely applicable by binding any biotinylated material and is currently utilized for in vivo application by others.

For testing the in vivo relevance of slanMo in psoriasis, we established an in vivo human psoriatic xenograft model by using immunodeficient NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice. The full engraftment of lesional psoriatic skin was observed within the first 10 days. At 21 days post transplantation we monitored the typical histopathologic and clinical features of lesional psoriatic skin. The high frequency of slanMo in these skin transplants supports our rationale that slanMo contribute to the proinflammatory immune response in the psoriatic skin transplants. We are currently targeting slanMo in skin transplants to induce their depletion. The effects and clinical benefits will be scored by histopathology and additional immunohistochemical stainings will reveal the overall immune cell distribution.

Taken together we created a therapeutic targeting and depletion strategy for human slanMo which is now being studied in a xenogenic psoriatic skin transplantation model. Studying the therapeutic potential of this new slan-based targeting strategy will foster our understanding of the psoriasis pathogenesis and the immunobiology of slanMo.

P082 | Increased expression of inhibitory checkpoint receptors in bullous pemphigoid

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Background: Bullous pemphigoid (BP) is the most common autoimmune skin blistering disease caused by specific autoantibodies against the proteins BP180 and BP230 within the dermal-epidermal junction. Dense blisters, erythema and wheals are the main clinical features of BP patients. Checkpoint molecules play a critical role in the maintenance of immune homeostasis by enhancing or inhibiting immune responses. In the last decade, targeting these checkpoint pathways has been increasingly used in the treatment of tumor patients, showing high efficacy in various tumor entities.

Methods: In order to investigate the role of co-inhibitory checkpoint receptors in the pathogenesis of BP, we analyzed skin sections of patients with BP (n= 17-19) and control subjects (n= 8-9) for expression of PD-1, Tim-3 and Lag-3 by immunohistochemistry. Furthermore, we measured serum level of PD-1 in BP patients and controls using a commercially available ELISA. We also compared serum levels of PD-1 with its expression in the skin.

Results: Investigating the number of PD-1-positive cells in skin sections, we observed a significantly increased expression in BP patients compared to controls (BP: 114.9 22.0 PD-1-positive cells/mm^2; control: 14.4 2.8 cells/mm^2; mean SEM). PD-1 expression in BP skin did not differ between patients with or without a dermal epidermal junction split/blister. Similarly, expression of Tim-3 protein was clearly enhanced in patients with BP (BP: 167.1 23.7 Tim-3-positive cells/mm^2; control: 42.5 6.3 cells/mm^2). In contrast to PD-1 and Tim-3, expression of Lag-3 was comparable between BP and controls (BP: 16.2 6.0 Lag-3-positive cells/mm^2; control: 6.9 4.3 cells/mm^2). In the serum, we found a tendency of enhanced PD-1 levels (not significant), correlating in part with its expression levels in skin sections.

Conclusions: Taken together, we report on increased expression of the co-inhibitory checkpoint receptors PD-1 and Tim-3, but not Lag-3, in BP, suggesting involvement of checkpoint receptors in the pathogenesis of BP. Our next experiments will focus on identification of specific cell types expressing checkpoint receptors and on elucidating the function of checkpoint receptors in BP. Interestingly, patients with tumors receiving antibodies against checkpoint ligands or receptors often also develop, as side effect, autoimmune processes, among those also bullous pemphigoid-like skin lesions.

P083 | Comparison of PDE4 expression in different chronic inflammatory dermatoses

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Chronic inflammatory dermatoses are widespread. It is therefore of great importance to understand the pathogenesis and progression of this conditions. Detailed understanding and comparison of the underlying molecular processes are of considerable interest to develop novel treatment options but also to reveal possible relationships between different skin diseases. Uncovering of similarities in the disease driving protein cascades open up the possibility to transfer already tested treatment options among the related skin conditions.

For this reason the aim of our study was to detect equally misregulated factors in five different chronic dermatoses (Psoriasis vulgaris, Atopic dermatitis, Lichen ruber, Pyoderma gangrenosum, Hidradenitis suppurativa). All of the examined skin conditions are auto inflammatory, immune driven diseases resulting from imbalanced interplay of pro- and antiinflammatory factors, e.g. cytokines and transcription factors. Proteins related to inflammatory signalling pathways are therefore best suitable for such comparative examinations. Since it is known that members of the Phosphodiesterase-4 (PDE4) family, due to over activation of proinflammatory NFκB signalling, play a key role in many different chronic inflammatory diseases, we selected all proinflammatory acting subclasses of the enzyme phosphodiesterase 4 (PDE4) and the downstream regulated proinflammatory cytokine TNFα for our comparative study. Protein occurrence was immunohistochemically investigated in paraffin embedded samples of inflamed skin areas from patients with Psoriasis vulgaris, Atopic dermatitis, Lichen ruber, Pyoderma gangrenosum, Hidradenitis suppurativa. As expected due to their inflammatory character we could show massive immune cell infiltration in the dermis of all examined conditions. Those infiltrates were highly positive for PDE4 A, PDE4 B, PDE4 D but negative for PDE4 C in all dermatoses. Furthermore TNFα was detectable across all samples. The comparability of the PDE4 expression pattern in Psoriasis vulgaris, Atopic dermatitis, Lichen ruber, Pyoderma gangrenosum and Hidradenitis suppurativa as well as the presence of TNFα in all conditions suggests on the one hand a relationship of all conditions and on the other hand comparable treatment options for all five dermatoses, e.g. via inhibition of TNFα or other proinflammatory products of NFκB signalling.

P084 | Upregulation of SERPINB3/B4 and S100A7-9 in hidradenitis suppurativa/acne inversa

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The serpin peptidase inhibitors (SERPINB3/B4) are known for their role in the dysregulated cell growth of squamous cell carcinoma cells, while the levels of calprotectin (S100A8/A9) are shown to be

increased in inflammatory conditions. In this study we investigate the expression of SERPINB3/B4 and S100A7-A9 proteins in hidradenitis suppurativa/acne inversa (HS). Comparative analysis of gene expression in the lesional vs perilesional skin from microarray data of 8 affected patients and 4 healthy individuals showed 705 statistically significantly upregulated genes and 482 downregulated (fold change >2, $p < 0.05$). In relation to the biopsies taken from control individuals, 994 genes were upregulated and 1046 downregulated. SERPINB3/B4, as well as S100A7/A8/A9, which were also highly expressed in lesional skin in comparison to perilesional skin, were selected for further investigation. Using qRT-PCR, the detected overexpression was validated. SERPINB4, S100A8/A9 were specifically overexpressed in the lesional skin with average fold changes of 18.5, 23, and 28.3, respectively. This finding was further confirmed at the protein level by immunohistochemistry. Our findings may implicate new pathways in the pathogenesis of HS, which are associated with interruption of essential cellular functions, e.g. cross linking of protein at the terminal differentiation of follicular keratinocytes.

P085 | Getting in touch with epidermal cell subtypes: a novel strategy to decipher the pathogenetic mechanisms in chronic inflammatory skin diseases

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A major feature of chronic inflammatory skin diseases e.g. psoriasis, hidradenitis suppurativa (acne inversa) or atopic dermatitis is the disturbed cellular function of epidermal keratinocytes. This includes an altered terminal differentiation and proliferation behavior of these cells, which is reflected by the development of characteristic skin lesions. Despite broad research attempts, the disclosure of pathogenic mechanisms underlying these diseases is a continuous challenge as respective key processes only occur in defined subpopulations of epidermal cells. As whole skin biopsy analysis does not allow an attribution to a certain cell type, we developed a purification strategy for obtaining different interfollicular epidermal cell subtypes suitable for downstream multiscale-omics analyses. The utility of our protocol was demonstrated by separation of keratinocyte subtypes from skin biopsies of healthy donors and non-lesional as well as lesional skin areas of psoriasis patients. The high suitability of our protocol was proved based on high quality and reproducible results obtained in subsequent downstream multiscaleomics analyses. In summary, the described method might be an appropriate tool to decipher the underlying epidermal subtype specific pathogenetic mechanisms in chronic inflammatory skin diseases.

P086 | Integrated miRNA and mRNA expression profiling in peri-lesional and lesional skin biopsies from psoriasis and psoriatic arthritis patients

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Psoriasis is the most prevalent pro-inflammatory skin disease with a substantial increased risk of arthritis. Lesional plaque development is characterized by a complex interplay between keratinocytes, immune cells and inflammatory disorders. Transcriptome profiling is commonly used as a biomarker for disease progression and monitoring of treatment effects. MicroRNAs (miRNAs) are short non-coding RNA species which are important post-transcriptional regulators of gene expression and play an important role during plaque development. To identify early molecular mechanisms during plaque development lesional, peri-lesional and non-lesional skin biopsies from 7 patients with psoriasis (PsO) and 7 patients with psoriatic arthritis (PsA) were analyzed using integrated mRNA-miRNA analysis. MiRNA profiling was assessed using the Nanostring nCounter technology and transcriptome profiling with RNA sequencing. In total, the expression of 28 miRNAs was identified to be differentially expressed across the gradient from non-lesional to peri-lesional to lesional skin with an at least 2-fold significant increase of 17 miRNAs and decrease of 11 miRNAs. Inverse correlation with 1929 genes across this gradient identified 139 miRNA-mRNA pairs. Most of these interactions were characterized by imbalanced keratinocyte function, immune response and fibrotic mechanisms which were already present in peri-lesional skin. Interestingly, none of these interactions can distinguish early plaque development in PsO from PsA patients. Our study revealed new functional insights in psoriatic skin lesions by combined miRNA-mRNA inverse correlation analysis. This approach can be used to identify early changes during disease progression and as a delicate mechanistic tool for differentiating treatment effects.

P087 | Female and male HS - two sides of a coin?

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Background: Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease which affects both genders.

Objectives: This study aimed to identify HS risk factors, comorbidities and impacts according to gender distribution, and to characterize gender-specific clinical manifestation and therapy in HS patients.

Methods: In the scope of this prospective study with ~400 HS patients, demographic, anamnestic and clinical data as well as laboratory parameters were collected and analyzed.

Results: There were no significant differences in age at disease onset and duration between female and male patients. Importantly, central obesity in female patients in contrast to acne vulgaris as well as a smoking habit with consumption of >20 cigarettes/day in male patients were identified as significant risk factors. Upon self-reported patient′s disease onset as well as described upon medical consultation, axillary sites in male and inguinal sites in female patients were most commonly involved. Female patients showed a significantly higher number of skin areas with inflammatory nodules, whereas fistulas, and accordingly, a higher Hurley score were observed in men afflicted with HS. Interestingly, there were no differences in the treatment regimen performed in the female or male subpopulation, although metabolic alterations, were more pronounced in male patients and female patients were found to suffer significantly more often from back pain. Furthermore, although both genders seemed to be similarly impaired in their QoL as assesses by DLQI scoring, significant differences were revealed in specific QoL aspects. Molecular analyses of lesional HS skin expression pattern between genders highlighted our findings.

Limitations: Data were partly obtained from self-administered patient questionnaires.

Conclusion: Significant differences in HS risk factors and comorbidities as well as clinical manifestation of the disease were found between the female and male patient population. Thus, consideration of the gender aspect for patient care and treatment decision is highly recommended.

P088 | Time course of mast cell infiltration and associated immune cells in a murine model of bullous pemphigoid

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Background: Bullous pemphigoid is an autoimmune disease characterized by cutaneous blisters and dermal inflammations, which are related to autoantibodies directed against hemidesmosomal proteins BP180 (COL17) and BP230. Within the inflammatory infiltrate of bullous pemphigoid, mast cells (MCs) are present besides eosinophils, neutrophils and other immune cells. The microenvironment potentiates the capacity of mast cells to amplify immune cell responses. However, this extent of MC function in autoimmune diseases is less understood. Thus, with the long-term goal of unraveling the role of MCs in bullous pemphigoid, we aimed, in the present study, to determine the time points of mast cell accumulation and the dynamics they interplay with infiltrating immune cells.

Methods: C57BL/6 mice aged 8 weeks were injected with 20 mg pathogenic rabbit anti-mCOL17 IgG, and observed for development of skin alterations over 12 days. Naive mice and mice receiving rabbit IgG served as controls. Skin biopsies were harvested on days 2, 4, 8 and 12, and analyzed for numbers of avidin+ MC, MBP + eosinophils, $Ly6G^+$ neutrophils, $CD3^+$ T cells and $CD11c^+$ dendritic cells using double immunofluorescence. To assess degranulation of MCs, cutaneous sections were stained with toluidine blue. Of each skin biopsy, 5 randomly selected images of the upper skin were acquired using a Keyence microscope at 200× magnification and analyzed with Image J.

Results: Overall, numbers of MCs were significantly increased in anti-mCOL17 IgG-injected mice compared to controls. MC counts showed a first peak on day 2, declined thereafter and strongly increased again on day 12. We detected a subepidermal linear alignment of MCs below the dermal-epidermal separation. Degranulation of MCs was noted throughout the course of the disease. Occasionally, eosinophils were found in close proximity to MCs, reaching peak numbers on days 4 and 8. In contrast, neutrophil infiltration peaked already on day 2 and persisted until day 12, whereas T cells showed high cell counts starting on day 4. We observed significant correlations between MCs and T cell numbers as well as between MCs and dendritic cell numbers.

Conclusions: Our results implicate MCs in the initiation and progression of bullous pemphigoid. Infiltration of MCs at late time points as well as their correlation with T cells and dendritic cells incriminates their role in the wound-healing phase. Thus, mediators released from MCs might also be involved in sustaining immune cell infiltration and thereby shaping the immune response. Our next studies will aim at investigating bullous pemphigoid in MC-deficient transgenic mice.

EPIDEMIOLOGY

P089 | Quality of life aspects of patients with psoriasis vulgaris, lupus erythematosus and urticaria

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Introduction: Diagnosis and treatments of patients with psoriasis vulgaris, urticarial or lupus erythematodes are often accompanied by distinct doctors in both outpatient and in-patient care. At least the chronic character of these diseases with recurrent symptoms and long-lasting treatments may advance to emotional and mental stress as well as to physical isolation. Furthermore lack of admittance and capability of the rural population to gain appropriate therapies may lead to false diagnosis or therapeutic oversupply by these patients. These circumstances does not only touch the patients' health but also the quality of life of these pa This situation does not only affect the health but also the overall quality of life of these patients'.

Material/methods: This study addresses the major factors underlying the patient's perceived health status and is based on a cross-sectional design. Questionnaire administration and clinical documentation takes place when patients were present at the outpatient clinic of the Department of Dermatology, University Hospital Regensburg. Key inclusion criteria: diagnoses psoriasis, urticaria or lupus erythematodes.

The questionnaires address skin-specific aspects as well as general aspects (activities of daily living, overall health-status) of quality of life. This study addresses patient-centered aspects of dermatological care for patients with psoriasis, urticaria and lupus concerning the patient's perceived health status (quality of life) in context of an analysis of a prospective study.

Results: The evaluation of the questionnaire showed, that the majority of patients consider it necessary to consult a medical specialist (here: dermatologist). The DLQI score was 8.3 by patients with lupus, 9.2 by patients with psoriasis, and 10.8 for patients with urticaria. The EQ-5D-5L showed a score of 61.6 for patients with a journey distance of >50 km and 66.3 for patients with a journey distance less than 50 km. The majority of all patients feel it very important that they are being treated by a medical specialist. This was more related to patients with a journey distance of >50 km.

Conclusions: In all 3 groups the patients considered it very important that the current medical care situation must be improved. No significant difference were observed in all 3 groups concerning the EQ-5D-5L questionnaire about problems in daily activities and bodily pain. Patients with an access road >50 km had a lower quality of life.

P090 | Prevalence of skin diseases on the fringe of the Munich Oktoberfest

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Background: Skin diseases are the fourth leading cause of nonfatal burden, affecting almost one-third of the world's population. In Germany, around 20 million cases are treated each year. However, real-life prevalence may be even higher since individuals, who do not consult a physician, are not considered in insurance database studies

Objectives: To examine the prevalence of skin diseases in a non-medical setting.

Methods: Visitors to the "Bavarian Central Agricultural Festival", at the outskirts of the Munich Octoberfest, were invited to participate in a cross-sectional study consisting of a free skin cancer screening and a paper-based questionnaire.

Results: Out of 2701 participants (1445 women, 1248 men), 1669 individuals (61.8%) suffered from any skin diseases. The most common diseases were nonmelanoma skin cancer (26.4%) and rosacea (24.3%). Moreover, 1.3% of all participants were affected by psoriasis, 7.3% by hand eczema and 1.3% by atopic eczema. Overall, the majority (80.0%) of people diagnosed with any skin disease was not aware of it before the checkup.

Conclusion: Due to a high prevalence, dermatological diseases indicate a significant socioeconomic burden. More information campaigns are needed to improve awareness of skin diseases.

GENETICS

P091 | Comparative genomics of mammalian keratins reveals loss of K1, K2, and K10 in the course of an evolutionary remodeling of the suprabasal epidermal cytoskeleton in fully aquatic mammals

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Keratins K1, K2 and K10 are the most abundant proteins of the human suprabasal epidermis where they form the cytoskeleton at the skin barrier to the environment. Here, we investigated the evolutionary conservation and loss of suprabasal epidermal keratin genes by comparative genomics. Publicly available genome sequences of representative species of all major phylogenetic clades of mammals were screened by bioinformatic methods. In contrast to the basal epidermal keratins K5 and K14, which are highly conserved among mammals, the suprabasal epidermal keratins K1, K2 and K10 have been inactivated in fully aquatic mammals of the phylogenetic clade Cetacea, comprising dolphins and whales. As all the abovementioned keratins were present in the last common ancestor of humans and cetaceans, these data suggest that dolphins and whales are natural knockout models for suprabasal epidermal keratins. Moreover, the functions of K1, K2, and K10 appear to be required only in a terrestrial environment. This study shows that the availability of genome sequences of a phenotypically diverse set of species allows new approaches for the comprehensive characterization of genes in complex biological contexts.

P092 | LEKTI is substrate of Transglutaminase-1: implication for a therapeutic topical replacement strategy for Netherton Syndrome

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Netherton syndrome (NTS) is a severe autosomal recessive ichthyosis caused by a deficiency of LEKTI encoded by the gene SPINK5. This serine protease inhibitor has 15 specific domains that are active inhibitors of various proteases of the skin.

We hypothesized that certain LEKTI domains are incorporated into the cornified cell envelope (CCE), thus providing a stable anchorage for this protease inhibitor.

Our in silico analysis of the overall protein revealed at least five consensus sequences that could serve as binding sites for transglutaminases (TGase) such as TGase 1 or TGase 3. Indeed, TGase activity tests on cryosections of human skin showed that biotinylated peptides of short LEKTI sequences were active as substrates. Using an *E. coli* based expression system we generated specific recombinant LEKTI domains (D6; D7) and a larger domain combination (D8/9) carrying such consensus sequences. Further in vitro experiments confirmed that specific recombinant domains of LEKTI are targets of TGase 1.

Our study calls attention to a probably underestimated pathophysiological link between the formation of the CCE by TGase-1 and its protection from serine proteases by specific domains of LEKTI. Considering the high need for an effective therapy (e. g. by topical protein substitution) our results may be highly relevant for its development.

P093 | Targeted resequencing of psoriasiform skin disease associated locus 1 (PSD1) in the CD18 hypomorphic psoriasis mouse model

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Psoriasis is a complex, chronic, multifactorial inflammatory skin disease characterized by four major manifestations: erythema, red crust, slivery scaly and skin lesions. We have previously shown that the PL/J mouse with a CD18hypo mutation resulting in a reduced expression of the common chain of β2 integrins (CD11/CD18) and spontaneously developed a skin disease that closely resembles human psoriasis. Interestingly, when backcrossed onto the C57BL/6J background no psoriasiform developed, suggesting that apart from the CD18 hypomorphic mutation a small number of modifier gene are additionally involved in the precipitation of the disease. A genome-wide linkage analysis identified susceptible loci on chromosome 6 and chromosome 10. Furthermore using a congenic approach, we identified a 9.0 cM fragment designated as psoriasiform skin disease associated locus 1 (PSD1) on chromosome 10 spanning D10mit126 - D10mit194 region harbouring genes which along with the CD18hypo mutation is responsible for development of the psoriasiform disease. To identify causal involvement of PSD1 locus in development of disease, we performed targeted and massively parallel sequencing in CD18hypo PL/J & C57BL/6J mice DNA samples. This approach identified the variants lying within exons, flanking regions and introns of PSD1 locus on chromosome 10. While analyzing the sequences of PSD1 locus, excluding CD18 based differences, heterozygous and intergenic variants, our results revealed 166 homozygous variants. Interestingly, out of these 166 homozygous variants, 11 variants are novel (no additional data known) and 155 variants lying within D10mit126 are already known and show the same nucleotide as C57BL/6J. Next we aimed to further validate and explore the possible role of these 11 is psoriasiform disease observed in CD18 hypo mice.

P094 | Insights into the shared aetiology of atopic dermatitis, asthma and hay fever

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Atopic dermatitis, asthma and hay fever are often observed within the same individuals, partly because of a shared genetic background. So far, fifteen shared genetic risk factors have been identified by single-disease genome-wide association studies (GWAS), but most remain uncharacterized.

We performed a GWAS (n=360 838) of a broad allergic disease phenotype that considers the presence of any one of these three. We carried out extensive follow up and enrichment analysis to understand biological consequences. Plausible target genes were defined by identification of coding variants, expression quantitative trait locus and publicly available functional data. The target genes were carried forward to identify enriched pathways as well as disproportional overexpression in different tissues and cell types exploiting publicly available data. Using integrative omics data we searched for cis expression quantitative methylation potentially modulating target gene expression by environmental risk factors.

We identified 136 independent risk variants ($P<3 \times 10^{-8}$) including 88 novel variants. Disease-specific effects were detected for only six variants, confirming that most represent shared risk factors. There were 244 likely target genes of risk variants, including 131 (54%) not previously implicated in allergic disease pathophysiology. We observed a significant enrichment of target gene expression SNP heritability

amongst immune cell subsets (e.g. BAFF$^+$ T cells, CD56$^+$ NK cells), with weaker but detectable effects in lung and skin. For 81 target genes we found CpG methylation that influence transcription independently of genetic effects.

Our results demonstrate that asthma, hay fever and eczema coexist to a large extent because they share many genetic risk variants that dysregulate the expression of mainly immune-related genes. Finally, our results suggest that environmental factors such as smoking might influence allergic disease risk through modulation of target gene methylation.

P095 | Absence of somatic mutations in linear localized scleroderma

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Linear localized scleroderma (LLS) is a rare connective tissue disorder characterized by chronic inflammation and massive accumulation of collagen. This then results in both hardening and thickening of the lesion leading to the affected areas to cave in from atrophy. The sharply delimited and linear lesions, can affect patients in areas throughout the body and more rarely the face in the clinical subtypes en coup de sabre and Parry-Romberg syndrome. This leads to terrible disfigurement that cannot be concealed. The disease affects mostly children and is limited in treatment options, which are most often unsatisfactory. The incidence of LLS in adults and children is 2.7/100'000 population per year.

Very little is understood about the condition in terms of both genetic and clinical aetiology. There is increasing evidence that LLS might be based on genetic alterations in affected tissues. Blaschko's lines are the patterns of cell migration and proliferation during embryological development. Multiple skin conditions have been shown to follow Blaschko's lines including LLS. Several of these diseases have been demonstrated to be caused by genetic factors such as a de novo somatic mutation causing a cutaneous mosaicism. Here we tested the hypothesis that LLS is caused by a somatic genetic mutation.

Blood and affected skin taken from 19 histologically confirmed LLS patients was Whole Exome Sequenced (WES). Library preparation and hybridization was performed using the SureSelectXT Reagent kits from Agilent Technologies. Sequence analysis was performed as per GATKv3.5. Somatic mutations were called using 3 somatic callers. All SNPs and indels were analysed and no rare and damaging mutation was found in common. 5 patients had damaging germline SNP mutations in PRSS3, a gene involved in metabolism and the immune system. No minor allelic frequency data is available for any of the PRSS3 mutations found so comparison with internal controls has shown 2 of 5 to be rare and 3 of 5 were present in some controls.

An analysis of the 3 somatic callers revealed that an increase in read depth (DP) allowed for a more sensitive detection of mutations at low allelic fraction. To this end 4 patients with similar lesions were chosen for deep sequencing (DP of >300). However, somatic analysis of these samples revealed no suitable causative mutation. CGH was performed on 3 patients to find large-scale chromosomal aberrations too large to be detected by WES. Samples were compared with 3000 control karyotypes. No rare aberrations were found in common between patients. A related work recently analysed three samples of LLS on the transcription level. No signal survived after correction for multiple testing. Taken together, our analysis revealed the absence of genetic mosaicism in LLS.

P096 | Uniparental inheritance of junctional epidermolysis bullosa through a novel mutation of the ITGA6 gene and trisomic rescue

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Background: Junctional epidermolysis bullosa (JEB) is a rare genetic blistering disorder due to damaging mutations in genes encoding for laminin subunits, integrins or collagen XVIIa. Blistering can be triggered by mild trauma to the area but can also arise spontaneously. Any organ with a mucous membrane is at risk for involvement. There is as of yet no cure. Treatment of the blisters, prevention of infection and correction of extracutaneous complications is the only clinical treatment for patients. JEB is autosomal recessive. In very rare cases, germline mosaicism and uniparental isodisomy have been reported. Here we investigate the genetic cause in a female newborn with skin aplasia, progressive junctional epidermolysis bullosa with pyloric atresia. An amniocentesis was conducted due to developmental abnormalities in the embryo, which detected low-level fetal mosaic trisomy of chromosome 2.

Methods: Prenatal amniocentesis, immunofluorescence mapping, transmission electron microscopy and genetic analysis were used for diagnostic evaluation.

Results: Immunofluorescence mapping showed a junctional split and absence of immunoreactivity for integrin á6. Sequence analysis of the ITGA6 gene on chromosome 2 revealed a homozygous frame-shift insertion, leading to a premature termination codon. This

mutation was found in a heterozygous state in the mother, but not in the father. Segregation analysis with chromosome 2-specific short tandem repeat (STR) markers exhibited exclusive maternal inheritance of chromosome 2, thus demonstrating evidence for uniparental disomy (UPD2) due to trisomic rescue. Whole exome sequencing was used to confirm the ITGA6 mutation and investigate any additional damaging homozygous mutations in chromosome 2 as a result of the UPD2. Interestingly, a damaging COL4A4 mutation was identified, which is however not a gene previously described in epidermolysis bullosa.

Conclusion: Full trisomy as well as high-level mosaicism would lead to spontaneous miscarriages or severe fetal malformations. Due to a very rare event of trisomy rescue, a uniparental disomy can lead to the manifestation of a recessive condition in case of mutation transmission by only one parent. This case demonstrates uniparental isodisomy caused a severe form of fatal junctional epidermolysis bullosa.

P097 | Prediction of gain-of-function and loss-of-function mutations using In Silico Bioinformatics Tools

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Introduction: Next generation sequencing (NGS) technologies can identify on average 20 000 missense variants per exome, out of which very few can be held responsible for causing the underlying disease. Therefore, it is very important to interpret the functional significance of these variants. A number of in silico bioinformatics tools have been already developed for the similar purpose, out of which CADD (Combined Annotation Dependent Depletion), SIFT (Sorting Intolerant From Tolerant) and PolyPhen2 (Polymorphism Phenotyping v2.0) are most commonly used. While SIFT and PolyPhen2 predict the deleteriousness of variants, CADD scores the variants based on a supervised machine learning approach (Support Vector Machine). There are also tools like Condel (CONsensus DELeteriousness score of nonsynonymous single nucleotide variants), which integrates the output of SIFT and PolyPhen2 along with other tools in order to assess the variant impact on protein function. The aim of this study is to develop a computational model based on these tools that can predict whether the variant causes a gain-of-function or loss-of-function at the protein level.

Material & Methods: A large number of variants was extracted from public database ClinVar, namely 522 gain-of-function (GOF) and 3350 loss-of-function (LOF) variants. All of these variants have supporting evidence of their functional significance in terms of literature texts, clinical reports and research projects.

Results: Our results show two peaks of mean Condel scores across GOF and LOF variants ($p < 0.05$) indicating a non-random difference. This difference along with the Condel score distribution over all the variants helped us to choose a cut-off score of GOF and LOF variants. The cut-off scores, in combination with CADD Phred scores, resulted in correct prediction of 71% GOF and 75% LOF variants.

Discussion: Therefore, these Condel cut-off scores can be useful to predict the functional significance of a missense variant. However, they should be used more as an indicator as they need to be interpreted with further evidence on pathogenicity.

P098 | Alterations of the composition of the extracellular matrix of integrin 3 deficient keratinocytes reflect basement membrane abnormalities in ILNEB

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αIntegrin α3β1 is widely expressed in epithelia at the cell-matrix and cell-cell interfaces. Loss-of function mutations in the integrin α3 gene (ITGA3) cause an autosomal recessive multiorgan disorder - interstitial lung disease, nephrotic syndrome and epidermolysis bullosa (ILNEB, MIM#614748). The molecular mechanisms of the complex disease manifestations are still unclear, yet abnormalities of the basement membranes are a common feature in the affected organs. Here, we performed a global unbiased analysis to detect changes in the abundance of soluble and deposited extracellular proteins secreted by integrin α3 deficient keratinocytes (A3-) compared to normal human keratinocytes (Co). Cells were SILAC labeled, and the soluble (medium) and insoluble (deposited) extracellular compartments were collected separately. Proteomic analysis was performed using experimental settings and bioinformatics data processing pipeline described before. In the deposited extracellular matrix (ECM) 167 proteins were identified, 34% (57) of them being regulated in A3- compared to Co. Of the 217 proteins detected in the soluble ECM, 26% (57) were regulated in A3-. Comparison with the results of gene expression studies revealed that regulation of ECM proteins was mainly on transcriptional level. To validate these findings and demonstrate that the observed regulations are the direct consequence of the absence of integrin α3, we induced the rescue of α3 in the A3- cells by stable transduction with retroviral particles containing the full-length human ITGA3 cDNA (A3-+A3). Using these cells in 2D and 3Dorganotypic co-culture (OTC) models, we demonstrate that, absence of the integrin α3 in keratinocytes switches the laminin-rich ECM to a fibronectin-rich ECM and significantly impacts the abundance of several ECM proteins including nephronectin and chondroitin sulfate proteoglycan. Furthermore, these proteins were similarly regulated in the skin, kidney and lung samples of patients with ILNEB, supporting the relevance of our findings in vivo.

P099 | Identification of the HLA locus and mitochondrial variants as genetic risk factors for Bullous Pemphigoid

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Bullous Pemphigoid (BP) is the most common autoimmune skin blistering disease. Although ample evidence suggests that BP is partially genetically determined, among others, by certain HLA haplotypes, IL-1β gene variants, and copy number variations of Fcγ receptor genes, a comprehensive genetic study of PD patients, including a genome-wide association study (GWAS), has never been conducted.

To this end, we performed the first GWAS for BP using the Illumina UK BioBank Axiom Array, involving a German cohort of 447 patients and 436 age- and sex matched controls. This study revealed a strong association of the HLA locus, with a subsequent gene enrichment analysis implying further additional variants.

Mitochondrial sequencing of the same German cohort revealed an association of variants in the two mitochondrially encoded tRNAs for Arginine and Threonine, which suggests a role for mitochondrial dysfunction in BP pathogenesis. This is supported by previous results from our group that identified a link between mitochondrial (dys)function caused by mtATP8 mutations and autoimmune blistering diseases.

A complementing analysis via RNA-Sequencing of perilesional skin biopsies of 13 BP patients also suggested an important role for mitochondria in autoimmune inflammatory disease modulation, through strong up regulation of numerous mitochondrial genes and corresponding down regulation of the mitochondrial regulator FOXO3.

Taking these findings together, we suggest that mitochondrial activity is a key factor in modulating the autoimmune response in BP. This novel association may open a new avenue in therapeutic modulation of the disease.

P100 | In vivo screening of functional novel oncogenic driver mutations in a Zebrafish melanoma model

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Our analysis of 615 melanoma exomes revealed 165 potentially interesting mutations whose functional consequences are only partially known. This project aims to perform functional in vivo screening of novel oncogenic enhancer mutations in a zebrafish melanoma model. The project outlines the creation of different transgenic lines of fish with a null p53 background coupled to overexpression of tumor oncogenes expressed on melanocytes and its comparison to the tumor incidence curves in order to determine genes with a functional relevance in melanoma progression particularly those involved in resistance. For this reason, the genes BRAFV600E, NRASQ61K, PIK3CAwt, PIK3CAH1047R, PIK3CAE545K, PTRF and STK11 were chosen based on the top hits in MelArray analysis previously done in the lab. To functionally test these candidate genes for the ability to accelerate melanoma transgenic zebrafish that over express these genes on a Tp53 mutant background is being observed. The targeted genes is being tested under 2 different promoters- MITF and SOX10. Genome editing techniques such as CRISPR-Cas 9 was used for knocking out tumor suppressor gene Tp53 alongside Gateway cloning technology that was used to over express oncogenes in order to create the transgenic lines.

Although targeted therapy and immunotherapy have revolutionized the treatment of melanoma, the importance of epigenetic factors targeting histones and histone modifiers in driving the behavior of melanoma is only starting to be revealed and provides significant opportunities to combat the problems of therapeutic resistance. Disruption of chromatin and altered expression of chromatin modifiers has been shown to play a role in melanoma development. It was observed that the family of methyltransferase KMT and demthyltransferase DNMT was most frequently mutated in the patient samples with BRAF/NRAS mutation status in the MelArray analysis. The zebrafish melanoma tumors resulting from our transgenic lines were induced with selection pressure by drug treatments to check if the difference in expression of KMT or DNMT can result in metastasis or resistant tumors in zebrafish.

Transplantation of mammalian melanoma cells into zebrafish larvae and adults offers a novel platform for melanoma gene discovery, cancer biology and small molecule screening. Therefore, in a second project, we used zebrafish as a xenograft model to test the effect of BRAF and MEK inhibitors in human melanoma cells positive for BRAF/NRAS mutation transplanted into zebrafish larvae in the presence or absence of PTRF, PIK3CA and STK11 genes. In order to establish the technique to make xenografts the behavior of 4 different melanoma cell lines- M121224, M130219, M980513 and WM793B was studied by labeling them with fluorescent DyeI and injecting them into wild type zebrafish yolk 1 day post-fertilization. The migrations of the cells was then monitored on a confocal microscope up to 4 days post injection.

Through the creation and analysis of several transgenic animals, one or more genes capable of accelerating/rescuing melanoma could be identified. This project provides a unique opportunity to interrogate the relationship between chromatin regulation and melanoma development.

HEALTH SERVICES RESEARCH

P101 | Association of DLQI and WLQ in patients with atopic dermatitis—results from the German AD Registry TREATgermany

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Background: Clinical registries may provide high quality evidence on the use and effectiveness of therapeutic interventions under real-life conditions. They are an indispensable prerequisite of evidence-based health care and translation of research evidence into clinical practice.

Methods: Initiated in 2011 and relaunched in 2016, the German Atopic Dermatitis (AD) Registry TREATgermany is the first registry of patients with moderate to severe AD worldwide. Adults with moderate-to-severe AD (current/prior systemic antiinflammatory treatment and/or objective SCORAD20) are prospectively followed over the course of at least 24 months. Employed treatment modalities and a broad set of physician and patient reported outcome measures are documented using validated measurement instruments to assess clinical disease severity (EASI, objective SCORAD), quality of life (DLQI), symptoms (POEM), global disease severity, as well as patient satisfaction and work limitations including presentism (WLQ). Herein, we present findings on the association of DLQI and WLQ of patients enrolled in the registry from June 2016 until September 2017.

Results: Overall, 201 individuals (mean age 43 years, 63% men) were enrolled at 19 recruitment centers. 74% of them were currently employed (76% of males, 70% of females, mean age 42 years). Employed persons had DLQI and WLQ scores of 10.47.0 points and 18.419.3%, respectively. Mean presentism, i.e. productivity loss while on the job, was substantial accounting for 9.5%. With coefficients of 0.393 and 0.350 WLQ and presentism scores significantly correlate with DLQI ($P<.000$). Bootstrapped regression models (corrected R©÷=0.15) showed that the limitations in coping with work requirements increase by 1.1% as DLQI increases by one point. If the subscales of WLQ are considered, it becomes apparent that lower quality of life due to AD is most strongly associated with limitations in the area of physical and performance requirements in general. Presentism decreases by 0.5% as DLQI increases by one point.

Discussion: Moderate-to-severe AD has substantial adverse economic impact with mean productivity loss of patients of almost 10%. Future analyses from TREATgermany will address the impact of innovative treatment modalities on quality of life and work productivity of patients with moderate-to-severe AD.

P102 | Prevalence and determinants of Psoriasis in a cross-sectional study of the elderly—results from the German AugUR study

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Background & Objective: There is a lack of knowledge on inflammatory skin diseases in the elderly. A recent review (1) showed in particular that there are only a few studies covering the prevalence of psoriasis in the aged population with wide variation in prevalence estimates (1% to nearly 19%). Determinants of psoriasis in the elderly have also been insufficiently investigated thus far. Therefore, the aim of this study was to investigate prevalence of and factors associated with psoriasis in an elderly population in Germany.

Material & Methods: We analyzed baseline data of the AugUR study (Age-related diseases: understanding genetic and non-genetic influences - a study at the University of Regensburg). AugUR (2) is a cohort study in a mobile elderly population conducted in Regensburg and surrounding areas (n=1133; median age: 76.7). Our focus was on the analysis of the prevalence, the role of comorbidities and lifestyle factors as well as self-rated health.

Median and range were computed for continuous variables as evidence for non-normality was considered. Differences were evaluated by Mann-Whitney-Wilcoxon test. To assess differences between people with and without psoriasis, the Pearson's Chi-square-test was used. All analyses were undertaken using SAS.

Results: A total of 5.48% study participants reported to ever have been diagnosed with psoriasis, among those 35.5% were female. Prevalence was highest in the group aged 80-84 years (7.8%), lowest in those aged 90-95 (0%). In our cross-sectional study, we found no conditions and comorbidities to be significantly associated with the presence of psoriasis (gender, body mass index, alcohol consumption, physical activity, self-reported physical condition, quality of life, diabetes, asthma, stroke, myocardial infarction, heart

weakness, stent implantation / bypass surgery). Only recent and former smoking tended to be more prevalent in patients with psoriasis: 56.45% in comparison to 43.83% in the group without psoriasis (P=.052).

Discussion: To our knowledge this is the first population-based study on psoriasis in highly-aged mobile elderly people. The prevalence estimate we found (5.5%) is higher than the estimate found in a German study using health insurance data (3) which showed a prevalence of psoriasis of 2.5%.

Furthermore, more men than women reported psoriasis.

Even if none of the lifestyle factors showed a significantly different frequency between people with and without psoriasis, there was a tentative association between (former) smoking and psoriasis which is in line with previous studies. Moreover, the self reported physical condition was far better in participants affected by psoriasis than not affected ones. This result might be biased due to other severe diseases in the latter group, though. The lack of association with any comorbidity was surprising.

References: 1) Hahnel E, Lichterfeld A, Blume-Peytavi U, Kottner J. The epidemiology of skin conditions in the aged: A systematic review. J Tissue Viability. 2017 Feb;26(1):20-8.

2) Stark K, Olden M, Brandl C, Dietl A, Zimmermann ME, Schelter SC, et al. The German AugUR study: study protocol of a prospective study to investigate chronic diseases in the elderly. BMC Geriatr. 2015;15:130.

3) Augustin M, Reich K, Glaeske G, Schaefer I, Radtke M. Co-morbidity and age related prevalence of psoriasis: Analysis of health insurance data in Germany. Acta Derm Venereol. 2010 Mar;90(2):147-51.

P103 | Baseline characteristics of patients with Atopic Dermatitis observed in the German AD Registry TREATgermany

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Background: For analysis and descriptive reporting, clinical registries document and manage longitudinal patient data regarding utilization and effectiveness of treatments as well as aspects of patient and quality management under real-life conditions.

Methods: The German Atopic Dermatitis (AD) Registry TREATgermany is a prospective multicentered registry. It emerged from TREATeczema and is part of the European registry family TREAT.

Adults with moderate-to-severe atopic dermatitis (current/previous anti-inflammatory systemic treatment and/or objective SCORAD≥20) are prospectively followed over at least 24 months. Objective clinical severity (EASI, objective SCORAD), disease symptoms (POEM), severity of pruritus and sleeping problems (VAS), flares, quality of life (DLQI) as well as patient and physician treatment satisfaction are assessed by validated measurement instruments, and treatments are documented. Here, we describe the baseline characteristics, treatment choices, and patient satisfaction of all patients included in the registry from June 2016 until September 2017.

Results: A total of 201 patients (mean age 4314 years, 37% female) were included in 19 recruitment centers with a mean objective SCORAD of 37.115.4 and a mean EASI of 12.610.6. Of these patients 55.5% had an early disease onset and 22.0% reported that they developed the disease in adulthood. The most frequent symptoms were skin dryness (82.0%) and pruritus (84.5%). 73.0% of the patients received systemic treatment. Oral glucocorticosteroids (59.5%) were the most frequently applied medication followed by cyclosporine (43.0%). Dupilumab, azathioprine, methotrexate, mycophenolate, alitretinoin, tralokinumab, secukinumab und leflunomide were also used in individual patients. Among patients with systemic treatment 54.8% received more than one treatment. Almost all patients were treated with topic agents (97%). Patients were fairly satisfied with medical supply (7.12.6; scale 0-10) and treatment (6.62.8; scale 0-10), however AD was poorly controlled (<4 of 12 weeks) in half of the patients and only in 5.5% completely controlled (>10 of 12 weeks).

Conclusions: This baseline analysis of the first 201 patients from TREATgermany provides valuable information about the usual treatment of adults with moderate-tosevere AD in Germany. It shows the effectiveness and utility of the current treatment, the high disease burden and with that the need for additional effective and safe treatment alternatives for long term control.

P104 | A novel machine-learning based approach to predict flares of psoriasis

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Psoriasis is a chronic inflammatory skin disease, characterized by periods of flareups and remissions. Multiple clinical risk factors and biomarkers can help to predict relapse of psoriasis, but optimal patient-friendly tools are still missing.

Psoracle is a novel approach to predict psoriasis flares that uses the most important clinical risk factors as biomarkers. Based on our own cohort of 1'800 psoriasis patients, we identified the major risk factors for flares such as irritation of the skin, dryness, missed psoriasis drugs and others. These data are now collected in an online questionnaire, which allows calculating the risk of the patient to develop a psoriasis flare.

To allow the prediction to become more accurate over time, a machine-learning algorithm has been implemented. It learns the clinical pattern of risk factors of patients who indicate that they currently have a psoriasis flare and adds up to the information of our previously analyzed cohort. This allows to compare each new patient to this pattern, which is constantly being updated, and therefore make each risk prediction a little more powerful and accurate than the last.

In seek of even more precise personalized prediction of the flare, we implemented an additional algorithm that includes serial data of returning patients. Learning what clinical risk factors and biomarkers correlate with relapse or remission in a given patient, enables to create a unique pattern for each returning patient and give predictions that are even more accurate.

Every patient response, entered in the online-questionnaire, contributes to the predictive power of the tool. This project will take time to collect thousands of patients' responses and the real value of the approach is going to manifest after thousands of users have entered real-life data. Today, Psoracle is a webpage-tool, but we aim to implement it as a smartphone application to make it approachable for even more patients.

P105 | Unmet digital health service needs in dermatology patients

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Digital health services, such as online health information and electronic doctor-patient communication are rapidly gaining acceptance in healthcare systems. Dermatology as an image-centric specialty is particularly well suited for telemedical services. However, dermatology patients' demands of electronic services remain largely unexplored. This study investigated patients' views in primary, secondary and tertiary referral centers.

In August 2017, 841 questionnaires were filled in by dermatology patients. 76.34% expressed interest in using digital healthcare services as part of medical consultations. There was no significant difference between female and male patients (78.93 vs. 76.21%). Comfort with digital health services was reduced in older patients (>50 years). 84.41% of all patients would complete their initial registration form electronically. Fewer patients were comfortable with sending pictures of skin changes to their doctors using email (40.89%) or mobile health applications (40.61%). Specific interest was indicated for arranging appointments online (90.80%) and electronically placed prescriptions (76.56%), rather than online learning videos (42.03%), actual online consultations (34.53%), and online explanations of medical procedures (27.19%). 65.37% of patients would pay for online consultations themselves, with patients between 35-49 years more willing to do so (72.17%).

Taken together, interest in electronic health services is high in dermatology patients. Our data suggests that readily understandable electronic services such as online arranged appointments and electronic prescriptions are of higher interest to patients than the current type of online consultations. Therefore, the full potential of teledermatology still remains to be tapped by newer, more attractive forms of services closely adapted to patients' demands.

IMMUNOLOGY

P106 | 12/15-lipoxygenase counteracts and resolves pemphigoid disease-like dermatitis

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"Resolution" is the active, programmed termination of tissue inflammation and the restoration of tissue homeostasis. It is presumably orchestrated by pro-resolving lipid mediators (SPMs). 12/15-lipoxygenase (12/15-LO) is a key enzyme in the biosynthesis of many pro-resolving lipid mediators (SPMs). The mechanisms of resolution of skin inflammation, such as in pemphigoid diseases has remained elusive. We have therefore addressed the role of 12/15-LO in the antibody transfer bullous pemphigoid-like epidermolysis bullosa acquisita (BP-like EBA) mouse model. In this model, pemphigoid disease-like dermatitis was pronouncedly aggravated and prolonged in 12/15-LO deficient (Alox15$^{-/-}$) mice when compared to wild-type mice. Moreover, elevated levels of DHA-derived SPMs including 10,17-DiHDHA, 17(S)- HDHA, and 14(S)-HDHA were found in lesional skin of WT mice, but not in skin of Alox15$^{-/-}$ mice, indicating that 12/15-LO counteracts skin inflammation and drives its resolution by biosynthesizing SPMs. In pemphigoid disease-like skin lesions, 12/15- LO expression was expressed in neutrophils, suggesting that neutrophils are critically involved in regulating the resolution of skin inflammation. Altogether, our results support the notion that 12/15-LO-derived SPMs are crucial to resolve of pemphigoid disease-like skin lesions in a timely manner. Hence, 12/15-LO-derived SPMs may be suitable as drugs in the treatment of pemphigoid diseases.

P107 | Monocyte recruitment and monocyte/platelet cluster formation in systemic sclerosis

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Introduction: Systemic sclerosis (SSc) is a connective tissue disorder with unknown ethology. A multitude of data have been reported in terms of fibroblast activation and the role of adaptive immune responses, but the specific contribution of innate immune cells in the early manifestation of the disease remain largely undefined. Apart from their central role in hemostasis and thrombosis platelets are important modulators of immune processes. It is known that platelets contribute to systemic sclerosis by e.g. production of profibrogenic TGF-ß and subsequent fibroblast activation. This projects aims to identify the distinct role of platelets in scleroderma and their potential to interfere with monocyte recruitment.

Methods: We studied two murine models of systemic sclerosis: Fra-2 mice which overexpress fos-related-antigen 2 under MHCI promoter control and therefore spontaneously develop vasculopathy and organ and skin fibrosis. The inflammatory response in Fra-2 mice peaks at week 7-9 with fibrosis setting in by week 12 leading to dermal manifestations and fatal pulmonary fibrosis with the endpoint in week 17 of age. Dermal sclerosis can also be induced by daily s.c. injection of hypochlorous (HOCl) acid over a period of 4 weeks leading to oxidative stress induced inflammation and subsequent collagen accumulation in the skin.

Results: Early vasculopathy (microangiopathy, vessel obliteration) and inflammation in Fra-2 mice was associated with increased levels of ($Ly6C^+MHCII^+$) inflammatory monocytes in blood and skin as well as enhanced Nur77 expression in myeloid cells which was not present in lymphocytes. This indicates that activation of myeloid cells/monocytes via Nur77 is an early event in SSc pathogenesis. In addition we found accelerated CD163 expression (in week 7-9) on inflammatory monocytes in Fra-2 mice further pointing towards enhanced monocyte activation in the inflammatory phase of fibrosis development. Blood analysis of Fra-2 mice revealed increased numbers of heterotypic conjugates between $CD11b^+$ myeloid cells and $CD41^+$ platelets (MPC). The conjugate formation started directly after birth and an accumulation of MPC correlated with disease severity and fibrosis development. By depleting platelets during the inflammatory phase of SSc development fibrosis outcome was significantly reduced. Fra-2 mice aged 3 weeks were injected once weekly with a monoclonal platelet depleting antibody against a common glycoprotein on platelets (GPIbα, clone 5A7) till onset of fibrosis in week 12. Mice were screened in week 15 for the accumulation of collagen in the skin (Goldner ′s trichrome staining). The skin thickness as well as hydroxyproline contents in skin were measured accordingly. Absence of platelets during the inflammatory phase led to reduced fibrosis development with regard to collagen accumulation and skin thickness. Interestingly, IL-4R/GPIbα-Tg mice in which the extracellular GPIbα domain is absent on platelets, the endothelium and monocytes revealed a significantly reduced cutaneous collagen accumulation (skin thickness) in response to SSc-inducing HOCl treatment. Elevated levels of CXCL-10, MCP-3, MIP-1α, CXCL-5 and RANTES in plasma preparations indicate a monocyte-/platelet- related inflammatory pathway.

Conclusion: Our study indicates that platelet -monocyte conjugation and GPIbα interaction of platelets and monocytes might be involved in inflammation and fibrosis development of SSC mice.

P108 | Disease activity in experimental bullous pemphigoid depends on IgG Fc N glycosylation

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Autoimmune bullous dermatoses (AIBD) are mediated by autoantibodies (Aab) directed against desmosomal and hemidesmosomal proteins. In bullous pemphigoid (BP), a subepidermal blistering dermatoses with antibodies directed against type XVII collagen (BP180), effector functions depend on binding of autoantibodies to BP180, activation of complement, and infiltration of inflammatory cells in the dermis. We and others have previously shown that interaction with Fcgamma receptors (FcγR) and glycosylation of anti-BP180 IgG are pivotal for blister formation. In other Aab-mediated diseases agalactosylated IgG Aab were associated with more severe disease, whereas galactosylation and sialylation of IgG Abs reduce their pathogenic potential. Here, we analyzed the glycosylation pattern of the IgG Aab of BP patients and age-matched controls by MALDI-TOF. We found a higher fraction of pro-inflammatory low-galactosylated Aab in BP patients (P=.070). Interestingly, anti-BP180 Aab were lower galactosylated compared to Aab against BP230, the other target antigen in BP with so far unclear pathogenic potential (P<.001). Also, anti-BP180 Aab in BP were lower galactosylated compared to anti-BP180 Aab in pemphigoid gestationis, a pemphigoid disease associated with pregnancy and to anti-desmoglein Aab in pemphigus sera (P≤.001; P=.021). In further experiments, high- and low-galactosylated anti-BP180 IgG was used to explore the functional relevance of glycosylation. In an in vitro model based on the release of reactive oxygen species (ROS) upon incubation of leucocytes with IgG, low galactosylation was associated with significantly higher ROS release. To analyze the effect of galactosylated IgG in vivo we injected anti-murine BP180 IgG with a terminal galactose residue into the ears of mice and measured the extent of lesion formation after 48 hours. In mice injected with IgG containing a terminal galactose residue, less skin lesions and lower myeloperoxidase activity in ear skin, a measure for the infiltration of neutrophils, compared to mice injected with agalactosylated anti-BP180 IgG (P=.031). Our data show that the pathogenic potential of Aab is modulated by their glycolisation pattern

and thus propose a novel therapeutic avenue for BP and other Aab-mediated diseases.

P109 | The role of circadian clocks in the development of epidermolysis bullosa acquisita

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A network of endogenous circadian clocks, coordinated by a central pacemaker in the hypothalamic region, regulates 24-h rhythms in skin physiology. The skin itself features a self-sustained, intrinsic clock which may affect our first-line defence against intruders over the course of the day. Epidermolysis bullosa acquisita (EBA) is a subepidermal blistering disease caused by autoantibodies to type-VII collagen. The treatment options are not yet considered satisfactory. Against this background, we aimed at investigating the role of circadian clocks in the development of EBA. We hypothesize that the migration of neutrophils into the skin, which controls the severity of autoantibody-induced tissue damage, is gated by peripheral circadian clocks. FACS analyses of peripheral blood mononuclear cells (PBMCs) were performed in healthy, male, C57BL/6 mice at defined circadian time points to characterize the diurnal regulation of PBMCs under basal conditions. Our data show that T cells, B cells, monocytes and neutrophils show circadian rhythmicity. Particularly neutrophils, the key players in the development of EBA, show significantly higher numbers in the morning than in the evening, whereas the activation of neutrophils is anti-phasic with higher levels in the evening. Ongoing experiments use the antibody transferinduced mouse model of EBA to investigate the impact of circadian timing on disease development. Autoantibodies were applied either in the morning or in the evening. First results show a significantly different outcome depending on the timing of the autoantibody injection. Additionally, we used two-photon microscopy to visualize neutrophil migration into the skin in lys-eGFP mice, possessing myelomonocytic cells labelled with green fluorescent protein. In this way we hope to gain new insights into EBA disease progression with the final aim to contribute to the development of novel chrono-therapeutic approaches.

P110 (OP01/02) | Topical treatment with a novel kappa-opioid receptor agonist ameliorates atopic dermatitis

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Opioids can induce potent analgesia without central adverse reactions by binding to peripheral opioid receptors. In this context, kappa-opioid receptor agonists (KORA) are of particular interest since they also exhibit anti-inflammatory properties and, in contrast to agonists of other opioid receptors, are not associated with visceral side effects. Kappa-opioid receptors (KOR) are expressed on keratinocytes as well as on immune cells and are up-regulated upon inflammation. Here, we investigated the anti-inflammatory potential of the newly developed KORA WOL071-007, belonging to the structural class of decahydroquinoxalines in murine and human immune cells as well as in a mouse model of atopic dermatitis (AD). In vitro, WOL071-007 down-regulated the expression of pro-inflammatory cytokines associated with AD development and progression, such as IL-4 or IL-13, in activated primary mouse and human immune cells. To avoid systemic effects a topical formulation was developed for clinical use in AD patients containing 1% of the KORA in an oil-in-water emulsion. Interestingly, the topical application of the WOL071-007-containing formulation significantly reduced ongoing AD-like skin inflammation in mice as evidenced by a decreased clinical score, the down-regulated infiltration of immune cells (particularly Th2 and mast cells) into lesional skin and the reduced erythema formation. Since AD is associated with severe itch we investigated whether WOL071-007, besides having anti-inflammatory capacities, also modulated itch. Notably, in mice with AD like skin inflammation, which were topically treated with the WOL071-007-containing formulation, the scratching frequency as well as the expression of the itch-associated cytokine IL-31 were dramatically decreased compared to controls. Of note, the antiinflammatory and anti-pruritic effects of WOL071-007 were mediated by binding to KOR since the new KORA did not ameliorate skin inflammation or itch in atopic KOR deficient mice. To investigate the safety and tolerability of WOL071-007 in humans we performed a phase 1B trial in AD patients, in which its anti-inflammatory and anti-pruritic potential was assessed as secondary objective, and interestingly, could detect a trend towards reduction in local SCORAD (scoring atopic dermatitis) index in WOL071-007-treated compared to placebo-treated patients. Together, our data clearly demonstrate that topical application of WOL071-007 significantly ameliorates ongoing skin inflammation and itch in a mouse model of AD as well as in AD patients, thus strongly suggesting WOL071-007 as a potential candidate for further clinical development.

P111 | Modification of the microbiome in psoriasis patients by systemic treatment with ustekinumab

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The skin is constantly exposed to environmental factors and forms a critical interface between the human body and the external

environment, prevents water loss, and represents the first barrier towards pathogens. But the skin also acts as an ecosystem providing physiologically and topographically distinct niches for microbial communities including bacteria, fungi or viruses. The majority of these microorganisms is neither pathogenic nor harmful to the host. However, it has been shown that commensal microbes influence the development and progression of various skin diseases including psoriasis. Next-generation-sequencing (NGS) approaches have been used to characterize the cutaneous microbiome in healthy skin compared to lesional skin from psoriasis patients revealing significant differences in the composition of the microbiota. The vast majority of bacteria present in healthy human skin belong to the four phyla Actinobacteria, Firmicutes, Proteobacteria and Bacteriodetes. However, a marked decrease in the total diversity of microbial communities, an overrepresentation of Proteobacteria and an underrepresentation of Actinobacteria has been observed in lesional skin from psoriasis patients compared to corresponding non-lesional skin from the same individual.

The aim of the present study was to investigate the impact of systemic treatment with ustekinumab, a fully human monoclonal antibody that selectively neutralizes the cytokines IL 12 and IL-23, on the composition of the microbiome in psoriasis patients. During the study the clinical response was evaluated by PASI-scores in 12 psoriasis patients treated with ustekinumab and 12 control subjects receiving cyclosporine. Moreover, skin swabs were collected at different time points (baseline, week 4, week 12 and week 24) after initiating the protocol for NGS analyses. To further characterize the systemic effects of ustekinumab, patients were asked to provide stool samples at the above mentioned time points.

Targeted amplicon sequencing of the 16S rRNA gene revealed an overrepresentation of Proteobacteria as well as an underrepresentation of Actinobacteria in inflammatory skin from psoriasis patients compared to non-lesional skin from the same individual. However, after treatment with ustekinumab the relative abundance of Proteobacteria decreased in many patients whereas we did not observe significant alterations in the relative abundance of Proteobacteria in swabs from cyclosporine treated controls suggesting that systemic ustekinumab might help to "normalize" the cutaneous microbiome in lesional skin from psoriasis patients. Interestingly, this effect was also detectable in stool samples, thus indicating that the impact of ustekinumab on the composition of the microbiome does not seem to be restricted to the skin. Worth mentioning, the alterations in the microbial communities in lesional skin and stool samples from patients treated with ustekinumab correlated with a reduced PASI score. Thus, it might be conceivable that successful treatment with ustekinumab could help to "normalize" the microbiota in psoriasis patients, which is associated with a reduction in the relative abundance of Proteobacteria.

P112 | A subset of long-lived memory T cells with proliferative potential resides in the skin of hematopoietic stem cell recipients

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Introduction: Due to lack of appropriate model systems, little is known about human T cell longevity, tissue residency and recirculation of peripheral tissues. Myeloablative conditioning therapy and subsequent allogeneic hematopoietic stem cell transplantation (HSCT) represent unique situations in the human body to study residency and repopulation dynamics of skin T cells. Therefore, we tracked T cells of 35 patients in the process of HSCT until one year after adoption of sex-matched (n=26) or sex-mismatched (n=9) donor stem cells.

Methods: Skin biopsies and peripheral blood were taken at ≤5 time points before and after HSCT and analyzed for T cell subtypes using lymphocyte homing molecules and residency markers. Isolated T cells were evaluated for their proliferative capacity upon T cell receptor (TCR) stimulation. Skin sections of patients after transplantation of sex-mismatched donor cells were assessed for chimerism by fluorescence in situ hybridization of X- and Y-chromosomes. Stained sections were processed using imaging acquisition and analysis software (TissueGnostics®).

Results: Upon myeloablative treatment, central T cells declined in skin and peripheral blood, while a subset of dermal cells expressing T cell residency markers ($CD3^+CD69^+CD103 \pm CCR7^-CD62L^-$) remained stable throughout all time points. This skin-resident subset displayed high proliferative potential after TCR stimulation. Overall, engraftment of skin leukocytes occurred at a slower rate than engraftment of peripheral blood mononuclear cells, normal cell numbers were only reached within ≥14 weeks post transplantation. Strikingly, chimerism analysis revealed that, unlike in peripheral blood, a skin-resident ($CD69^+CD103\pm$) population of dermal T cells of the recipient coexisted with donor T cells months after engraftment.

Conclusion: We have identified a long-lived and remarkably resistant population of dermal T cells which may present an important therapeutic target in persistent and relapsing inflammatory skin conditions.

P113 | Cells of the myeloid lineage act as early drivers of dermal fibrosis

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In systemic sclerosis (Scl), cellular mechanisms involved in the pathophysiology of cutaneous and organ fibrosis are highly complex. Dermal fibrosis is characterized by disproportionate accumulation of collagens and other extracellular matrix substances. The involvement of innate immune cells and the sequence of inflammatory events in the early phase of the disease is still unknown.

In our study, we used hypochlorous acid (HOCl) to chemically induce oxidative stress in the skin and trigger dermal fibrosis development. HOCl was daily injected intradermally for a period of 4 weeks. Furthermore, we analyzed Fra-2 mice which spontaneously develop a progressive cutaneous and organ fibrosis due to an overexpression of fos-related-antigen 2 under MHCI promoter control.

Histological analysis of HOCl-mice showed a significant increase in dermal thickness and a prominent increase in collagen fibers and total collagen levels accompanied by disorganized architecture at day 28, visualized by staining for H&E, Goldner's trichrome and PicroSiriusRed. Furthermore, we observed enhanced numbers of α-SMA$^+$ cells during the early phase as a hallmark for profound myofibroblast activation in HOCl-treated skin. Flow cytometric analysis of HOCl treated skin demonstrated an early cellular infiltrate at day 7 with significantly increased percentages of activated myeloid cells (CD11b$^+$MHCII$^+$) and inflammatory monocytes (CD11b$^+$Ly6C$^+$). To analyze the functional role of the CD11b$^+$Ly6C$^+$ myeloid cells during the early phase of HOCl-induced Scl, we used antibodies against CD11b and Ly6C or isotype controls, respectively. Absence of myeloid CD11b$^+$ and Ly6C$^+$ immune cells led to a reduction of HOCl-induced skin thickening at days 7 and 14 which became statistically significant at day 28. In addition, we found enhanced levels of IL-3, TNF-α, IL-5 and CxCL5 in the plasma of HOCl mice, indicating inflammatory processes driven by monocytes/macrophages.

Fra-2 mice exhibit a dermal fibrosis development at weeks 12-17 which manifests in dermal thickness and a prominent increase of total collagen levels (H&E, Goldner's trichrome, hydroxyproline assay). Analysis of inflammatory cells in the skin by flow cytometry revealed a significant increase of CD45$^+$CD11b$^+$ myeloid cells and activated monocytes (CD11b$^+$MHCII$^+$) at weeks 3-5 in Fra-2 mice compared to control animals. Further characterization of "M1" like (CD11b$^+$iNOS$^+$) and "M2" like (CD206$^+$CD301b$^+$) cells during the early phase of cutaneous fibrosis demonstrated an increased inflammation mainly driven by CD11b$^+$iNOS$^+$ and to a significantly lesser extent by CD301b$^+$CD206$^+$ myeloid cells. At later time points (at week 9 and 15), we did not observe significantly difference between the percentage of CD11b$^+$iNOS$^+$ and CD301b$^+$CD206$^+$ cells in the skin, indicating a mixed M1/M2 infiltration in Fra-2 mice.

In conclusion, our study demonstrates that CD11b$^+$ myeloid cells are the predominant immune cell subset in the early inflammatory phase in chemically induced and Fra-2 mouse models of dermal fibrosis. Further characterization of the myeloid subpopulation is important to identify therapeutics strategies in fibrosis.

P114 (OP05/01) | Glucocorticoids promote intrinsic human Th17 differentiation

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Although Th17 cells are critical to host protection at epithelial barriers, they can play a pathogenic role in inflammatory and autoimmune diseases. Glucocorticoids (GCs) are therapeutically used to suppress undesired inflammation, but the common persistence or even increase of Th17 responses in GC-treated patients led us to investigate the effect of GCs on Th17 development. We found that GCs trigger a Th17 differentiation program in naïve human CD4 T cells in the absence of antigen presenting cells or exogenous cytokines. GC-induced Th17 cells expressed IL-10 and their development was associated with an inhibition of Th2, and particularly Th1 differentiation. GC induction of Th17 differentiation was not linked to increased IL-1β, IL-6 and TGF-β, yet GCs downregulated IL-2. Consistently, the addition of exogenous IL-2 prevented the GC-induced increase in Th17/Th1 ratios. Also, IL-2/IL-2R blocking enhanced Th17/Th1 ratios, altogether suggesting a mechanistic role for IL-2 regulation in GC-induced Th17 differentiation. Functionally, supernatants of GC-differentiated T cells were superior to those of non-GC-differentiated T cells at inducing antimicrobial peptides and pro-IL-1β in keratinocytes, despite decreased IFN-γ and increased IL-10. Finally, GCs also favored Th17 over Th1 among memory T cells from blood and skin. Altogether, our data define GCs as human Th17 polarizing factors.

P115 | STAT1/STAT3 imbalance determines the clinical phenotype of chronic mucocutaneous candidiasis

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Background: Chronic mucocutaneous candidiasis (CMC) is clinically characterized by a defective clearance of the yeast Candida albicans. CMC represents a heterogeneous group of disorders attended by an impaired Th17 response. Several monogenetic defects have been identified to cause CMC. While the most frequent CMC form is a result of heterozygous autosomal-dominant gain-of-function (GOF)

mutations in signal transducer and activator of transcription (STAT) 1 (STAT1-CMC), there are also syndromic CMC entities, such as autosomal-dominant hyper-IgE syndrome (STAT3-HIES).

Objective: CMC patients with gain-of-function STAT1 (STAT1-CMC) and loss-of function STAT3 mutations (STAT3-HIES) were immunologically characterized to unravel the poorly understood pathomechanisms of CMC.

Methods: STAT DNA-binding activity during Th cell polarization was assessed using TransAM. Cytokine production in PBMCs was determined by flow cytometry and ELISA after anti-CD3/CD28 or Candida albicans stimulation.

Results: STAT1-CMC PBMCs showed a significantly higher STAT1, but a slightly reduced STAT3 activity than PBMCs from healthy subjects. STAT3 activity in STAT3-HIES was significantly reduced. The altered STAT1/STAT3 balance in STAT1-CMC and STAT3-HIES patients resulted in an impaired Th17 polarization with a reduced presence of Th17 cell subsets and an attenuated secretion of interleukin (IL)-17A and IL-22. While induction of Th17 and Th22 cell differentiation was possible in STAT1-CMC patients to a variable degree, it was impossible to overcome Th17 and Th22 defects in STAT3-HIES patients.

Conclusions: Our findings imply a less persistent impairment in Th17/Th22 cell differentiation in STAT1-CMC patients compared to STAT3-HIES patients.

P116 (OP04/06) | Type-17 immunity controls Malassezia-mediated skin infection

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The constant exposure of our epithelial surfaces to microbes is important for immune homeostasis, but also bears a constant threat to microbial invasion and disease. The skin microbiota comprises not only bacteria but also fungi, of which Malassezia spp. are by far the most prominent. There is accumulating evidence that Malassezia is involved in the development and/or exacerbation of various skin disorders including pityriasis versicolor, dandruff, seborrhoeic eczema and atopic dermatitis. The causal relationship between the fungus and these pathologies remains however unclear. To gain knowledge about the complex interplay between Malassezia and the skin immune system, we established a novel experimental model of Malassezia skin infection in mice. This has allowed deciphering the cellular and molecular mechanisms that control the fungal growth on the skin. Topical application of Malassezia spp. onto barrier-disrupted murine skin results in a robust immune response to the fungus characterized by skin thickening, infiltration of inflammatory leukocytes and the local production of cytokines and antimicrobial peptides. Infected mice develop a robust Th17 response in the draining lymph nodes and induce local production of type 17 cytokines in the skin. Consistent with this, we found that Malassezia-specific memory T cells in healthy human individuals that respond to the commensal fungus belong predominantly to the Th17 subset. Finally, we demonstrate that the IL-17 pathway is critical for fungal control because mice deficient in IL-17 production are unable to prevent fungal overgrowth. Together, our results demonstrate a critical and so far unrecognized role of type 17 immunity in keeping the balance between the skin commensal Malassezia and the mammalian immune system. This is reminiscent of what is known about the IL-17 pathway in the control of other fungal commensals such as Candida spp. and highlights the relevance of IL-17 in host protection at barrier tissues, in contrast to its pathological potential when dysregulated.

P117 | Neutrophil extracellular traps in Schnitzler's syndrome

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Introduction: Urticarial autoinflammatory diseases such as Schnitzler's syndrome (SchS) are characterized by the accumulation of neutrophils at skin sites of inflammation. The role of neutrophils in the pathogenesis of SchS, however, remains ill-defined. Recently, neutrophil extracellular traps (NETs) were shown to regulate IL-1ß-mediated inflammation in autoinflammatory disease familial Mediterranean fever (FMF). NETs are web-like structures of decondensed chromatin, histones and antimicrobial peptides released by neutrophils during NETosis. Here, we assessed the role of NETs in the pathogenesis of SchS.

Methods: Immunofluorescence co-staining of myeloperoxidase (MPO) and subnucleosomal complex (Histone 2A, 2B, chromatin) was performed on paraffin sections of lesional skin of patients with SchS (n=9), chronic spontaneous urticaria (CSU) (n=5) and healthy controls (n=10). Blood neutrophils from SchS (Canakinumab treated patients n=9, untreated patients n=3) and healthy controls (n=12) were isolated and NET formation was induced by phorbol 12-myristate 13-acetate (PMA) for 80, 100 and 130 minutes. Also, NET formation of control neutrophils by symptomatic SchS and FMF sera as well as healthy control sera and sub-threshold PMA doses was studied. Samples were stained (immunofluorescence co-staining of DAPI and subnucleosomal complex), and microscopic images were analyzed by ImageJ.

Results: Immunofluorescence co-staining revealed marked NET formation in lesional skin of all SchS patients but near absence of NETs in CSU patients. Correspondingly, PMA-stimulated blood neutrophils from the majority of Canakinumab treated as well as untreated

SchS patients showed higher NETosis rates over time compared to healthy control neutrophils with significant difference (P<.024) after 130 minutes of PMA-stimulation for Canakinumab treated patients. Moreover, co-stimulation of healthy control neutrophils by SchS serum and PMA disclosed a trend to more NETosis as compared to control sera and PMA.

Conclusion: Our results suggest that neutrophils contribute to the systemic inflammation in SchS via the induction of NETosis in lesional skin and peripheral blood. The relevance of these findings should be further explored in greater patient numbers.

P118 | Evaluation of potential novel biomarkers in immune mediated diseases

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Development of targeted therapy and immunotherapy, directed at specific pathological pathway molecules and cell markers, has resulted in an increased need for predictive and prognostic biomarkers in immune-mediated diseases. In many tumors such as melanoma predictive as well as prognostic (LDH, S100) biomarkers are integrated in the therapeutic guidelines as well as to monitor the benefit of therapy in regard to stage of disease and disease progress.

In a previous study, comparing protein levels in responders and non-responders of immune checkpoint treated patients, we identified a panel of discriminatory potential biomarkers using a quantitative proteomic analysis of plasma samples. Two candidates of this training set could already be validated in additional assays such as ELISA. Herein, Afamin levels showed significantly different regulation pattern in plasma of malignant melanoma patients compared to healthy controls.

Interestingly, the human vitamin E-binding glycoprotein Afamin is a pleiotropic protein involved in various disease states including different types of carcinoma such as gastric, thyroid or ovarial cancer. In addition, Afamin also showed to be associated with insulin resistance and incidence of DM Type II. Therefore, in the present work, we analyzed the dynamics of Afamin and its relevance in patients with autoimmune syndromes and other chronic inflammatory diseases such as psoriasis in comparison to our previously obtained data in melanoma patients.

To conclude, preliminary data revealed elevated levels of Afamin in the plasma of patients with psoriatic disease. Though the expression did not correlate to PASI score or to the existence of psoriasis arthritis, therapy with different immunomodulatory agents changed Afamin levels in peripheral blood of psoriasis patients. The detailed role of Afamin in psoriasis in the complex immunological interplay is currently under investigation.

P119 | Sensitive and specific assay for the serological diagnosis of anti-laminin 332 mucous membrane pemphigoid

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Next to BP180 (type XVII collagen), laminin 332 (Lam332) is the most relevant autoantigen in mucous membrane pemphigoid (MMP) targeted in about a quarter of MMP patients. Lam332, a glycoprotein important for the structural integrity of the dermal-epidermal junction, is a heterotrimer consisting of the alpha 3, beta 3, and gamma 2 chains. Exact diagnosis of anti-Lam332 MMP is important since nearly 30% of these patients develop solid cancers. However, so far no detection system for serum anti-Lam332 autoantibodies was commercially available. In this study, a cell-based indirect immunofluorescence (IF) test based on the eukaryotic expression of recombinant Lam332 (heterotrimer and single chains) in the human cell line HEK293 was developed. The novel assay was subsequently probed with a large number of MMP (n=246) including sera from anti-Lam332 positive MMP patients (n=93) defined by the clinical phenotype of predominant mucosal lesions and Western blot reactivity with Lam332 secreted by cultured human keratinocytes and Lam332-unreactive MMP patients (n=153) with antibodies staining the epidermal side of salt-split skin by indirect IF microscopy and/or reactivity against BP180 and/or BP230. Furthermore, sera from patients with bullous pemphigoid (n=20), pemphigus vulgaris (n=20), non-inflammatory dermatoses (n=22), and from blood donors (n=100) were included. Sensitivities with the heterotrimer (77%), the alpha 3 (43%), beta 3 (41%) and gamma 2 (13%) chains were obtained with a specificity of 100% for each substrate. Any combination of the single chains did not increase the sensitivity of the test using the heterotrimer. Sensitivity reached 88% when only the 80 MMP sera with anti-Lam332 antibodies detected by Western blotting and reactivity with the dermal side of human salt-split skin were included. Using FITC anti-human IgG spiked with FITC anti-human IgG4 as secondary antibody instead of FITC anti-human IgG4 antibody alone, six additional anti-Lam332 MMP sera could be recognized which did not exhibit anti-Lam332 reactivity by Western blotting using extracellular keratinocyte matrix. Applying the novel cell based IF test, a significant correlation of the anti-Lam332 IF titer with the disease activity was observed. The novel IF-based assay using the recombinant heterotrimeric Lam332 as a substrate for IF will greatly facilitate the serological diagnosis of anti-Lam332 MMP and thus help to identify the patients at risk of malignant tumors.

P120 | N-terminal autoantigen trimming may explain epistasis between HLAC* 06:02and ERAP1 variants in psoriasis risk

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NH2-terminal trimming by endoplasmic reticulum aminopeptidase 1 (ERAP1) generates the appropriate length of peptide antigens for binding to and presentation by HLA-class I molecules. ERAP1 polymorphisms result in different peptide trimming specificities and activities. Genetic interaction between ERAP1 variants and HLA class I alleles determines the genetic risk for various HLA-class I-associated inflammatory diseases. Epistasis between HLA-C*06:02 and ERAP1 variants also affects psoriasis risk but the mechanisms of these gene interactions remained unknown. In psoriasis, HLA-C*06:02 mediates an autoimmune response against melanocytes through autoantigen presentation. We have used an HLA-C*06:02- presented melanocyte autoantigen, ADAMTS-like protein 5 (ADAMTSL5), and an ADAMTSL5-specific Vα3S1/Vβ13S1 T-cell receptor (TCR) from pathogenic psoriatic $CD8^+$ T cells to determine the role of ERAP1 variants in psoriasis pathogenesis.

Our data show that ERAP1 variants increasing psoriasis risk efficiently generate the antigenic ADAMTSL5 peptide from NH2-terminally elongated ADAMTSL5 peptide precursors. Instead, a protective ERAP1 variant reduced the availability and immunogenicity of the antigenic ADAMTSL5 peptide presumably through overtrimming and peptide destruction. Other peptide ligands derived from natural human proteins and recognized by the Vα3S1/Vβ13S1-TCR due to TCR polyspecificity were not processed by ERAP1 from peptide precursors into antigenic peptides of appropriate length. Thus, using a proven psoriatic autoantigen from melanocytes and a pathogenic psoriatic TCR, these experiments provide direct evidence that gene-gene interaction between ERAP1 and HLA-C*06:02 affect the risk for psoriasis through differential trimming of a psoriatic peptide autoantigen. They furthermore demonstrate that different ERAP1 affinities for precursor peptides finally determine if autoantigens can be generated from the parent proteins in $CD8^+$ T-cell mediated autoimmune diseases. Accordingly, our results provide essential insights into the pathomechanisms of autoimmune diseases beyond psoriasis.

P121 | Multi-parameter flow cytometric profiling of T cell populations in psoriatic patients

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Psoriasis vulgaris is a T cell mediated skin disease with a variety of T cell populations implicated in disease development. T cells contribute to a chronic inflammatory environment in the skin, causing hyperproliferation of keratinocytes, which eventually leads to characteristic erythematous squamous plaques. To get a better understanding of how T cell populations in the peripheral blood are composed, we compared the number and percentage of regulatory T cells (Tregs) and conventional T-helper cells, including a panel of activation markers between healthy donors (n=7) and psoriatic patients (n=19) and between psoriatic patients before (n=19) and after (n=9) a systemic treatment, with (mainly) anti- TNF-alpha biologicals. For our study, we recruited psoriatic patients with a PASI score above 10, in order to see a significant improvement after treatment and to be able to compare the course of disease with immunological parameters. With the help of chemokine receptor expression and additional parameters, we characterized different T cell subsets in the blood that may be involved in the pathogenesis of psoriasis. Within $CD3^+CD4^+$ conventional T-helper (Th) cells we distinguished in Th1 ($CD25^{low}CXCR3^{high}CCR4^-CCR6^-$), Th2 ($CD25^{low}CXCR3^{low}CCR4^{high}CCR6^{high}$), Th17 ($CD25^{low}CXCR3^{high}CCR4^{high}CCR6^{high}$) and Th22 cells ($CD25^{low}CXCR3^{low}CCR4^{high}CCR10^{high}$) as well as in regulatory T cells ($CD25^{high}CD127^{low}FoxP3^{high}$ Treg), as the latter ones play an important role in inhibiting autoreactive T cells. Psoriasis treatment had no significant effect on Th1, Th2 and Th22 cells, but unexpectedly, we found that the number of Th17 cells (in 80 mL whole blood) increased from an average of 1.5 million cells before therapy to 3.9 million cells after therapy, which is almost the same level of cells that we observed in healthy donors (3.3 million cells). According to these results, the percentage of Th17 cells slightly rose after treatment of the psoriatic patients. Interestingly, the number of Tregs expressing the skin homing receptor cutaneous lymphocyte antigen (CLA) dropped from 430 000 before therapy to 180 000 cells as well as the percentage of CLA positive Tregs decreased from 27% before to 6% after treatment, reaching similar average values of Tregs in the blood of healthy donors. This flow cytometric study will help to identify a strategy to sort T-helper cells and Treg from patients at different disease stages for RNA sequencing. With this technique we intend to identify molecular targets which are regulated by certain biologicals in psoriasis.

P122 | The simultaneous siRNA-mediated downregulation of PD-1 and CTLA-4 to improve CAR-T cell immunotherapy of melanoma

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Chimeric antigen receptor (CAR)-T cells have already been used successfully in clinical trials for adoptive therapy of several types of

malignancies. The majority of the patients showed a beneficial response, highlighting the therapeutic effectiveness of this therapy. It is a challenge, however, that the efficiency of the engineered T cells is negatively influenced by inhibitory receptors on T cells, with programmed cell death protein 1 (PD-1)-receptor and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)-receptor representing the two major and well-investigated ones. Triggering of these inhibitory receptors may cause inhibition of T-cell activity resulting in tumor progression.

A novel strategy to overcome this problem is a combined transfection of the T cells with small-interfering RNAs (siRNAs) to downregulate the two inhibitory receptors PD-1 and CTLA-4 in addition to a mRNA coding for a CAR recognizing the surface antigen melanoma-associated chondroitin sulfate proteoglycan (MCSP) using RNA electroporation.

Cell-surface staining for PD-1 and intracellular staining for CTLA-4 showed that the expression of either one single receptor or both receptors was efficiently suppressed on the CAR-T cells after transfection with siRNAs when compared to CAR-T cells electroporated with negative control siRNA. PD-1 and CTLA-4 expression on CAR-T cells was downregulated approximately 50% and 30%, respectively. The simultaneous siRNA-transfection showed no influence on CAR expression of the engineered T cells. Functionality assays were performed after stimulation of the CAR-T cells with PD-L1- and CD80-transfected melanoma cell lines. While cytokine production in CAR-T cells transfected with both siRNAs was similar to CAR-T cells electroporated with negative control siRNA, a higher cytokine production was observed in CAR-T cells transfected with either PD-1 or CTLA-4 siRNA only. However, all CAR-T cell conditions showed a higher cytotoxicity.

Taken together, it is feasible to generate optimized CAR-T-cells by simultaneous transfection of CAR-encoding mRNA and siRNAs in order to downregulate the inhibitory receptors PD-1 and CTLA-4. Our data indicate an improvement of the in vitro functionality of these CAR-T cells and thus, this strategy could represent a novel method to enhance CAR-T cell immunotherapy of cancer.

P123 | The relevance of CMV reactivation in immunocompromised patients suffering from chronic inflammatory skin diseases

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Reactivation of latent CMV infection is a serious complication in immunocompromised patients, dramatically impairing clinical outcome. This might be in particular true for patients with chronic skin diseases fulfilling two major prerequisites of CMV reactivation in the human organism, namely ongoing inflammation and therapy-induced immunosuppression. As efficient therapies are available to control CMV replication, early detection and therapy of CMV reactivation is relevant to reduce long-term hospitalization and health costs.

The aim of this project was to elucidate the role of a potential reactivation of CMV infection in chronic skin diseases which require long-term immunosuppressive treatment.

In a retrospective study, we included a total number of 48 patients, suffering from a chronic skin disease, whose lesions did not improve or even worsened under immunosuppressive treatment (18 chronic ulcers/pyoderma gangrenosum, 21 bullous autoimmune diseases, 9 skin lymphomas). Formalin-fixed paraffin-embedded tissue was examined for the presence of CMV DNA by PCR, which could not be detected in bullous autoimmune diseases (n=21) and skin lymphomas (n=9), but in 1/18 chronic ulcers/pyoderma gangrenosum (5.6%). Next, within the framework of a small prospective study (n=29) we analyzed the seroprevalence of CMV as well as the presence of CMV DNA in lesional skin in patients that had been diagnosed with a chronic skin disease and in whom long-term immunosuppressive therapy had been initiated. 21/29 patients (72.4%) were seropositive for anti-CMV-IgG, as compared to the seroprevalence in the general population which ranges between 50% and 65%. Anti-CMV-IgM, indicating primary infection or reactivation of latent CMV infection, could be detected in 5/29 patients (17.2%), thereof one patient (A) was diagnosed with pyoderma gangrenosum, one (B) with pemphigus vulgaris and one (C) with ulcers. In addition, CMV DNA was detected in lesional skin biopsies of patient A and C. Despite being treated with high-dose steroids, and specific therapy regimens in addition (infliximab in A and rituximab in B), lesions worsened. In all three patients, treatment with ganciclovir was initiated leading to profound improvement of skin lesions and/or health status. Apart from that, CMV DNA could be detected in lesional skin of one patient who did not show anti-CMV-IgM. Taken together, we found CMV DNA in 3/19 lesional skin biopsies (15.8%).

Our study showed that the seroprevalence of CMV is elevated in patients suffering from chronic inflammatory skin diseases under immunosuppressive treatment as compared to the general population. Besides, the presented cases highlight that awareness of the phenomenon of CMV reactivation and prompt antiviral treatment might speed up improvement of health status also in immunocompromised patients with chronic inflammatory skin diseases. Ongoing research will focus on the functional role of CMV and CMV specific T cells in the context of chronically inflamed skin.

P124 | The role of the hair follicle in antibody transfer-induced epidermolysis bullosa acquisita

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Hair follicles undergo life-long cyclical transformations called the hair follicle cycle which consists of three phases: anagen (growth), catagen

(regression) and telogen (resting) phase. Moreover, during hair follicle cycling there is a repetitive construction and degeneration of the immune privilege which protects the hair follicle from being attacked by the immune system. Epidermolysis bullosa acquisita (EBA) is a severe autoimmune skin blistering disease characterized by autoantibodies against type VII collagen. Using the antibody transfer-induced mouse model of EBA we want to investigate the role of hair follicles in the development of EBA using hairless mice compared to the appropriate littermate controls. Hairless mice fully undergo hair follicle morphogenesis but hair follicles never enter the hair follicle cycle leading to a bald phenotype of these mice. Instead of functional hair follicles remaining cysts and a hyperplasia of sebaceous glands can be found in the skin accompanied by immune cell infiltration. In three separate experiments we see that the clinical score defined by the percentage of affected body surface area is significantly higher in the littermate controls compared to hairless mice. Using immunohistochemical analyses we can show IgG deposition along the dermo-epidermal junction. Further, we can also detect deposition of complement component 3 (C3) along the basement membrane overlaying the IgG deposition. Ongoing experiments will elucidate the role of blood vessels in the course of the disease development by immunohistochemical analyses using an anti-CD31 antibody staining for endothelial cells. Furthermore, we investigate neutrophils and T cells using immunohistological staining for both CD3 and Ly6G. Moreover, we want to investigate the role of the immune privilege for the development of EBA using immunohistochemical and immunofluorescence stainings for different markers which characterize the immune privilege. Our preliminary results show that the hair follicle seems to play a role in the disease development of EBA in mice.

P125 | Adipose tissue precursor cells sense and shape their environment at the wound site

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Mesenchymal Stem Cells (MSCs) are endowed with the capacity to sense environmental cues and to generate an integrated adaptive response in the interest of tissue homeostasis and repair. So far it is, however, largely unexplored how MSCs sense their environment and how they mount an adaptive response to shape the function and activation state of distinct immune cells during tissue injury. In the present study, we wished to investigate how MSCs adaptively regulate neutrophils function under conditions of wound infection, where MSCs suppression of neutrophil functions would be detrimental. Here, we evaluated the adaptive response of adipose derived MSCs (AD-MSCs) on activated neutrophil functions in the presence and absence of pathogen-associated molecular patterns (PAMP) such as bacterial lipopolysaccharide (LPS) mimicking an infectious wound environment. Of note, LPS-treated AD-MSCs substantially augment neutrophil activation resulting in an increased neutrophil extracellular trap (NET) formation and increased ROS production as opposed to MSCs suppression of activated neutrophils under "noninfectious" conditions. To further explore whether toll like receptor-4 (TLR-4) present on MSCs surface, is involved in the adaptive response of AD-MSCs, we specifically silenced the TLR-4 receptor gene employing specific siRNA. Our results show that TLR-4 silenced MSCs upon LPS treatment failed to activate neutrophils, subsequent NET formation and ROS production, indicating a causal role for TLR-4-dependent sensing LPS, subsequent signaling and shaping the adaptive response. RNAseq analysis, RT PCR, antibody arrays, and factor specific ELISA of AD-MSCs cultured in the presence or absence of LPS uncovered GCP-2 and IL-8, which are known to recruit and activate neutrophils. Collectively, we identified the mechanism underlying the master role of MSCs in the control of infectious cues and tissue integrity. Our data may even hold promise to be therapeutically exploited for the benefit of patients with difficult-to-treat and/or infected wounds.

P126 | Modifying melanoma immune microenvironment by heterologous prime-boost vaccination with adenovirus and Modified Vaccinia Ankara virus vectors

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Introduction: Multiple vaccine dosing is usually necessary to prime and boost humoral and cellular immune responses against the target antigen. Administration of the same antigen by using different viral vectors termed heterologous prime boost has been experimentally applied against infectious diseases such as HIV, malaria, tuberculosis and certain malignancies such as prostate cancer. Here we have developed a novel immunization strategy against melanoma consisting of adenovirus and Modified Vaccinia Ankara (MVA) mediated heterologous prime-boost vaccination towards gp100 and TRP1 melanoma antigens coupled with adoptive cell transfer (ACT) of transgenic $CD8^+$ and $CD4^+$ T cells targeting the respective antigens.

Materials & Methods: MVA and adenovirus vectors were engineered to ectopically express GP100 ($CD8^+$ T cell) and TRP1 ($CD4^+$ T cell) epitopes fused to mCherry Reporter and eYFP Reporter respectively(MVA-PMTP and AD5-GTY, respectively). The functionality of the vectors was first tested in vitro by co-culturing gp100 and TRP1 transgenic $CD8^+$ and $CD4^+$ T cells with transduced mouse splenocytes followed by IFN ELISA. Immunizations against established HCmel12 melanomas and in healthy B6 mice were carried out by priming the expansion of transferred $CD4^+$ and $CD8^+$ T cells with Ad5-GTY followed by MVA PMTP mediated boost 14 days later. CRISPR-Cas9 technology was used to generate Trp1 knockout HCmel 12 melanoma cell lines.

Results: Ad5-GTY and MVA-PMTP were able to stimulate PMEL CD8$^+$ T cells and TRP1 CD4$^+$ T cells in vitro. Both cell populations expanded efficiently in tumor free mice after Ad5-GTY priming. T cell expansion was efficiently boosted in the contraction phase with MVA-PMTP immunization in healthy but not HCmel12 melanoma bearing mice, where only PMEL CD8$^+$ T cell expansion was observed. Interestingly, intra-tumoral route of administration of MVA booster vaccine was found to be therapeutically more efficacious than intra-peritoneal route in CD4$^+$ T cell monotherapy suggesting local, beneficial effects in the tumor microenvironment. Trp1 knockout HCmel 12 melanomas were resistant to Trp1 CD4 T cell therapy indicating that CD4 T cell therapy requires target antigen expression by the melanoma cells.

Discussion: We have successfully generated adeno- and MVA vectors for priming and boosting of CD4 and CD8 T cell responses, respectively. Our results indicate that CD4$^+$ and CD8$^+$ T cell responses can be boosted in absence of melanoma, but in the tumor bearing hosts TRP1 CD4$^+$ T cell boosting was inhibited possibly as a result of tumor induced changes in the phenotype of the transferred TRP1 CD4$^+$ T cells

P127 | Combined anti-tumor immune responses of slan$^+$ monocytes and natural killer cells in malignant melanoma

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The concept of cancer immunosurveillance represented by a complex and multilayered interaction between immune cells and malignant tumor cells has provided the basis for very potent immunotherapy treatments of cancer. In our studies we focus on the interplay between NK cells and 6-sulfo LacNAc positive (slan$^+$) cells. Both cells type were previously identified in melanoma metastasis and are capable of initiating strong anti-tumor responses. slan$^+$ cells were previously identified as dendritic cells by functional means. However, we would now like to call them slan$^+$ monocytes based on the transcriptomic signature shared with monocytic cells and with reference to the new ontogeny driven nomenclature. The interaction of slan$^+$ monocytes with NK cells results in an increased tumor directed cytotoxicity as well as in high level production of proinflammatory cytokines such as TNF-α, IFN-γ, and IL-12.

Here, we address the question whether the cytokine milieu generated by co-culturing these two cell types can arrest the growth of tumor cells and lead to senescence induction in melanoma cells. To this end, we incubated melanoma cell lines with supernatants from slan$^+$ monocyte/NK cell co-cultures. Melanoma cells treated with supernatants showed a severely reduced proliferation rate as measured by reduced 3H-Thymidine incorporation and cell numbers. Most importantly, we observed the induction of p21 (CDKN1A) but not p16 (CDKN2A) in melanoma cell lines as a result of treatment with pro-inflammatory cytokines. Additionally, growth arrested cells exhibited increased senescence-associated β Galactosidase staining, a common marker for non-proliferative, senescent cells.

Taken together, our data demonstrates that slan$^+$ monocytes and NK cells in addition to a direct cytotoxic response can also mediate immune responses that drive melanoma cells into senescence.

P128 (OP01/05) | IL-17C: checkpoint in innate skin immunology

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Inflammatory skin diseases are frequent and have a major impact on patient's quality of life. A deeper understanding of regulators of misguided skin inflammation will lead to the development of novel therapeutic concepts. While the role of IL-17A as a key cytokine of inflammatory skin diseases is well known, other members of the IL-17 family such as IL-17C remain poorly investigated. In this study, we investigated cellular sources, triggers and intracellular signalling pathways as well as the function of IL-17C in inflamed skin. In a screening approach, we detected high numbers of IL-17C positive cells in diverse inflammatory, autoimmune and infectious skin diseases. Sources of IL-17C were keratinocytes, neutrophils and mast cells, but not adaptive immune cells. In primary human keratinocytes, IL-17C was induced by stimuli such as IL-1β, Flagellin and TNF-α via substantial upregulation of p65, phospho-p65 and IBα in the cytosol and nucleus. Furthermore, expression of IL-17C transcripts was dependent on NfB and ERK1/2. As intracellular signalling leading to IL-17C production is similar to pathways observed for TNF-α induction, we next investigated whether there is a functional synergism of IL-17C and TNF-α. In fact, stimulation with IL-17C led to enhanced expression of antimicrobial peptides in primary human keratinocytes, and this effect was synergistically potentiated by co-stimulation with TNF-α. Furthermore, cell-free supernatant of keratinocytes stimulated with IL-17C enhanced the migratory potential of neutrophil granulocytes to a level comparable to CXCL8. To assess the relevance of IL-17C in a complex model of human disease, we finally cultured human skin biopsies of psoriasis and atopic dermatitis with an IL-17C neutralizing antibody. Compared to untreated controls, neutralization of IL-17C led to a significant downregulation of pro-inflammatory cytokines and antimicrobial peptides (e.g. IL-36G, DEFB4A), demonstrating the crucial role of IL-17C in human inflammatory conditions. Taken together, IL-17C is broadly expressed in human skin pathologies, is induced by innate immune stimuli via activation of NFB and forms a pro-inflammatory feedback loop with TNF-α. Neutralization of IL-17C is a promising therapeutic strategy of inflammatory skin diseases.

P129 | Differential induction of ATF3 and HO-1 in myeloid cells and keratinocytes via Dimethyl fumarate or Cyclosporine A

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Most inflammatory skin diseases such as psoriasis, lichen planus or atopic dermatitis are characterized by a hyperplasia of the epidermis. Although in these diseases the inflammatory environment could promote proliferation, in these diseases proliferation is controlled and only rarely leads to cancer. Activating transcription factor 3 (ATF3) is one key factor which controls and regulates proliferation. ATF3 is a dual function protein as it suppresses pro-inflammatory IL-6 and IL-8, but also acts as a tumor promoter by the suppression of p53 which leads to an acceleration of the cell cycle. To dissect the bi-modal role of ATF3 in a cell type specific manner, comparing myeloid cells with keratinocytes, we pharmacologically induced ATF3 and analyzed its influence on cytokine expression and secretion. Since some inflammatory skin diseases can be treated systemically with Cyclosporin A (CsA) or Dimethyl fumarate (DMF) we used those drugs for the induction of ATF3. Interestingly only CsA treatment increases the risk of non-melanoma skin cancer, while DMF treatment does not increase tumor incidence. In the present study, we showed that ATF3 is induced in PBMCs by DMF, while CsA is the most prominent inducer of ATF3 in keratinocytes without enhancing HO-1 transcription. Further we could show that LPS treatment elevates IL-1β and IL-6 and weakly ATF3 transcription in PBMCs. While transcription of both cytokines is elevated, LPS treatment mediates IL-6 secretion while only a low amount of IL-1β is secreted. We could also show that the treatment with DMF dampens LPS-induced transcription. Taken together, our results shed light into the different carcinogenic potential of CsA and DMF, which both target ATF3. Collectively our data demonstrate that CsA strongly induces pro-carcinogenic ATF3 in keratinocytes, whereas ATF3 induction by DMF in myeloid cells acts antiinflammatory.

P130 | Mast cells modulate antigen-specific CD8 T cell activation during viral infections

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Mast cells (MCs) are important for innate and adaptive immunity and known to be involved in immune responses against bacterial infections. In contrast, little is known about their role in viral infections. Here, we investigated the role of MCs in host responses to lymphocytic cytomeningitis virus (LCMV), specifically their role in the activation of anti-viral CD8 T cells. In a well-characterized LCMV murine infection model, MC-deficient (Kitw-sh/Kitw-sh) mice displayed reduced frequencies of LCMV specific CD8 cells as compared to wild type control mice at day 8 post infection. Furthermore, we used an in vitro LCMV infection model, where bone marrow derived MCs were co-cultured with LCMV specific CD8 T cells. After 3 days of LCMV infection, bone marrow derived MCs induced the upregulation of extracellular activation markers such as CD25, CD69 and CD44 as well the downregulation of CD62L on CD8 T cells. Moreover, bone marrow derived MCs induced the increased of IFN-g and TNF-a production at day 3 post infection. These results suggest that MCs can function as non-professional antigen presenting cells and may directly modulate CD8 T cells responses during viral infections. A better understanding of the impact of MCs on CD8 T cell responses may help to improve antiviral immunity and to modulate and ameliorate inflammatory responses during viral infections including skin viral infections.

P131 | Effects of cold atmospheric plasma (CAP) on human peripheral blood mononuclear cells (PBMC)

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Introduction: Plasma is termed the fourth state of matter that is artificially generated by subjecting gas to an electromagnetic field. Medical interest in plasma further arouse as cold atmospheric plasma (CAP) was discovered enabling investigations within mild physiological temperatures (<40°C at the point of application) thus allowing in vitro and in vivo studies. CAP consists of excited atoms, electromagnetic fields, several types of radiation (e.g. UV-light, visible light, infra-red radiation) and free radicals. Free radicals, among them reactive oxygen- and nitrogen species (ROS/RNS) are presumed to play a major role in modulating cells. Nevertheless the precise effects differ dependent on the type of source gas and technical design of the generator (e.g. plasma Jet, Dielectric barrier discharge, glow discharge, Microwave plasma) as well as on treatment parameters like total treatment time.
Due to the ability of CAP to deactivate microorganisms, cause cell detachment, and cause death in cancer cells, it has been object of research in wound therapy, skin regeneration, dentistry, disinfection of surfaces and instruments as well as in oncology.
Previous studies demonstrated positive effects of CAP on wound healing due to promoting proliferation of eukaryotic cells and inducing lethal effects on prokaryotic cells. Immune cells such as peripheral blood mononuclear cells (PBMC) have a major contribution in wound healing. As a part of anti-tumor therapy CAP induces DNA changes, influences mechanisms of cell death, affects adhesion and motility.
Yet the specific underlying functional mechanisms of CAP are still elusive and require further investigation. Therefore, in this study, we aim to evoke predictable and standardized effects of CAP on PBMC within given conditions.
Materials & Methods: Stimulation of the cells was realized by direct and indirect treatment at different exposure times and distances to CAP microwave-plasma source. For direct treatment, cell cultures

were immediately subjected to the plasma effluent for different treatment times, whereas indirect treatment implied the application of CAP to the culture medium only in which PBMCs were re-suspended afterwards. Following the stimulation, the cultures were incubated at 37°C, 5% CO_2 for 3 and 6 days and cell analysis was performed afterwards. Cellular changes regarding composition, viability, phenotype and function were evaluated via flow cytometry, cytometric bead array and metabolic activity was demonstrated by MTT- Assay.

Perspective: Interpretation of the data should support a better understanding of the effects of cold atmospheric plasma on human PBMCs. Moreover the acquired knowledge should encourage further research about cold atmospheric plasma to broaden the clinical application spectrum. As CAP has multipotent beneficial effects on human PBMCs, it could be an interesting therapy for multiple medical indications involving immune mediated conditions

P132 (OP03/04) | Lack of regulatory T cells leads to development of pathogenic anti-BP230 autoantibodies and blister formation

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Peripheral tolerance provided by regulatory T cells is crucial to prevent the development of autoimmune diseases. Scurfy mice lack regulatory T cells due to a mutation in the foxp3 gene and develop different autoimmune diseases. Scurfy mice have strong Th1/Th2 prone T-cell mediated inflammation in the skin and show high levels of autoantibodies against skin proteins in sera and skin. In scurfy sera we detected autoantibodies against different autoantigens like BP180, Laminin 332, Collagen VII, which are associated with different types of autoimmune blistering diseases. Examination of scurfy skin shows the presence of subepidermal blisters and acanthosis. Together with the deposition of autoantibodies in the skin, we demonstrate that scurfy mice develop the phenotype of autoimmune blistering diseases.

We produced monoclonal antibodies from scurfy mice via hybridoma generation and screened them for their reactivity to skin proteins. One monoclonal antibody (20B12) showed reactivity to the basal membrane zone of murine and human skin. Mass spectrometry analysis revealed BP230 as the antigen of this IgG autoantibody. We confirmed the identified antigen by indirect immunofluorescence staining on BP230- expressing HEK293 T cells in comparison to non-transfected or BP180-expressing control cells. Injection of the anti-BP230 antibody 20B12 in neonatal wildtype mice showed in 70% of mice a subepidermal blister formation after 48 hours.

In further experiments we isolated $CD4^+$ T cells from scurfy and wildtype mice and transferred them into nude (nu/nu) or RAG knockout ($RAG^{-/-}$) mice. $RAG^{-/-}$ mice, which lack mature T and B cells, did not show any autoantibody production after transfer of scurfy $CD4^+$ T cells. Injection of autoreactive $CD4^+$ T cells of scurfy mice into nu/nu mice, which have B cells but no T cells, resulted in a similar panel of autoantibodies as in scurfy mice themselves. There was no induction of autoantibodies if $CD4^+$ T cells from WT mice were transferred into nu/nu mice. We detected blister formation in 90% of the nu/nu mice, which had received scurfy $CD4^+$ T cells. No blister formation was observed if WT $CD4^+$ T cells were injected into nu/nu mice. In summary, we show that a defect in peripheral tolerance leads to pathogenic autoantibody production with the ability to induce blisters in scurfy mice. Furthermore the injection of anti-BP230 autoantibody was sufficient to induce blister formation, therefore BP230 should be considered to be a major antigen in Bullous pemphigoid.

P133 | Activation of the lymphocyte function associated antigen 1 (LFA-1) is critical for the stable adhesion of $slan^+$ monocytes recruited via CD16 by intracapillary immune complexes

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There are pathologic conditions where immune complex (IC) deposition causes Fc receptor-dependent inflammatory lesions, such as lupus nephritis or vasculitis. A cell population directly recruited from the blood flow by IC are 6-sulfo LacNAc ($slan^+$) cells. These cells express CD16 for the interaction with IC and have functional features of dendritic cells. They are now called $slan^+$ monocytes, which is in line with their monocytic progenitor and the most recent nomenclature of mononuclear phagocytes. $CD16^+$ $slan^+$ Mo were previously reported to be recruited and activated by intracapillary IC in human lupus nephritis.

To investigate whether the integrin LFA-1 is required in the ICs-mediated $slan^+$ Mo recruitment, we applied a perfusion assay-based approach. Monolayers of dermal microvascular endothelial cells were preincubated with an endothelial cell specific antibody to mimic deposited IC. The arrest functions of purified $slan^+$ Mo was measured under physiological flow conditions. By time-lapse video microscopy and blocking strategies with monoclonal antibodies and LFA-1 inhibiting small molecules such as lovastatin we demonstrate that rolling is induced by the engagement of immobilized antibodies with CD16 and that firm adhesion requires activation of LFA-1.

Collectively, our results show that LFA-1 is required for the recruitment of $slan^+$ Mo to the IC deposited at the site of vasculature. Understanding these critical nuances of recruiting inflammatory cells allows to specifically immunomodulate certain IC mediated inflammatory and/or autoimmune diseases. Our results also underscore the rationale of previous immunomodulatory therapeutic

strategies of using the LFA-1 specific mAb efalizumab as well as current strategies that employ the LFA-1 inhibiting small molecule lovastatin.

P134 | Altered frequency of myeloid-derived suppressor cells (MDSC) and regulatory T cells (Treg) in peripheral blood of patients with psoriasis

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Psoriasis is one of the most common, chronic T cell-mediated inflammatory skin and joint diseases affecting up to 3% of the general population. It is known that keratinocytes and lymphocytes play an important role in the pathogenesis of psoriasis, but little is known about the significance of MDSC and Treg, both of which exhibit regulatory functions in immune responses. The aim of our study was to analyse and determine the number and function of blood-derived MDSC and Treg in psoriasis. We performed a comparative analysis of the frequency of these circulating regulatory cell populations in psoriatic patients and in healthy controls. A total of 111 patients with different types of psoriasis and 23 controls were included in the study. The frequency of MDSC and Treg subsets was measured using flow cytometry, and the results were analysed according to type of therapy and severity of disease. Severity of disease was assessed using the PASI score (Psoriasis Area and Severity Index), which showed a range of 0 to 45.8 points. The frequency of $CD14^+/CD124^+$ monocytic MDSC (M-MDSC) and $CD15^+/CD14^-/CD11b^+$ polymorphonuclear MDSC (PMN-MDSC) in psoriatic patients with systemic therapy was comparable to healthy controls. Patients with systemic therapy had significantly decreased numbers of $CD14^+/CD124^+$ M-MDSC compared to those with local therapy. Interestingly, their percentage increased with increasing PASI score in patients with systemic therapy. The frequency of PMN-MDSC was increased in patients with systemic therapy compared to topical treatment. The mean percentage of $CD15^-/CD14^+/CD33^{high}/HLA\text{-}DR^{low}$ M-MDSC and Lin- $(CD3^-/CD15^-/CD14^-/CD19^-)/HLA\text{-}DR^{low}/CD11b^+/CD33^+$ early-MDSC (e-MDSC) was clearly elevated in psoriasis compared to controls. The frequency of $CD15^-/CD14^+/CD33^{high}/HLA\text{-}DR^{low}$ M-MDSC and e-MDSC positively correlated with increasing PASI scores. The percentage of $CD25^+/Foxp3^+$ Treg in $CD3^+/CD4^+$ cells was not different in patients compared to healthy controls, it increased, however, with increasing PASI scores in those with systemic therapy. Psoriasis patients with local therapy tended to have a low frequency of $HLA\text{-}DR^+$ Treg compared to controls, an effect that was abrogated when systemic treatment was administered. The percentage of activated $GARP^+$ Treg in patients with systemic therapy appeared to be comparable to controls, both, however, tending to be higher compared to patients with only local therapy. The frequency of activated $GARP^+$ Treg increased with increasing PASI score in both therapy groups. In summary, our results indicate that the frequencies of MDSC and Treg in peripheral blood of psoriatic patients differ and depend on the severity of disease. In addition, systemic treatment appeared to "normalize" these effects to the level of healthy controls. More studies are needed to better understand the regulatory mechanisms contributing to control of psoriasis activity.

P135 | Deficiency of the G protein-coupled receptor GPR109a alters the phenotype and function of dendritic cells

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It was shown that short chain fatty acids (SCFA), bacterial products from fermentation of fiber, are involved in protection against inflammation in the colon and the skin via induction of regulatory T cells (Treg). One of the most relevant receptors for SCFA is the G protein-coupled receptor GPR109a/HCA2. To get further insight into the mechanisms by which Treg are induced via HCA2 signaling, we utilized $HCA2^{-/-}$ mice. As dendritic cells are critical in maintaining the balance between Treg and Th17 cells, we investigated the phenotype of bone marrow derived-dendritic cells (BMDC) from $HCA2^{-/-}$ mice. Quantitative real time PCR revealed that IL-6 and IL-17, cytokines which promote Th17 cells and suppress the development of Treg, were significantly upregulated in BMDC of $HCA2^{-/-}$ mice in comparison to wild type (WT) BMDC. WT BMDC expressed the aldehyde dehydrogenase Aldh1a1 which allows the conversion from naïve T cells into Treg, whereas this expression was significantly reduced in $HCA2^{-/-}$ mice. Accordingly, IL-10, a relevant promoter of Treg, was downregulated in $HCA2^{-/-}$ mice. All this together suggested that BMDC from $HCA2^{-/-}$ mice might be defective in inducting Treg, thus confirming that HCA2 signaling might be crucially relevant for Treg development. To prove whether this alteration is functionally relevant, BMDC from $HCA2^{-/-}$ and WT mice were pulsed with the hapten TNBS and subcutaneously injected into naïve recipients which were challenged 5 days later with TNCB. To our surprise upon injection of $HCA2^{-/-}$ BMDC the sensitization response was not enhanced (as supposed due to the reduced number of Treg) but significantly reduced in comparison to the reaction upon injection of WT BMDC, indicating that DC and not Treg are responsible for these effects. As initiation of an immune response is dependent on antigen uptake followed by processing and presentation to T cells, we studied the capability of antigen uptake of $HCA2^{-/-}$ BMDC in an antigen uptake assay using FITC-conjugated OVA as a model antigen. FACS analysis revealed that $HCA2^{-/-}$ BMDC were less potent in taking up FITC-OVA compared to WT BMDC. The reduced antigen uptake might explain why the sensitizing capacity of DC lacking HCA2 is reduced despite the absence of Treg. Taken together, these findings indicate HCA2 as a crucial receptor during antigen presentation which might essentially influence the outcome of an immune response.

P136 | UV-B irradiation of the skin improves experimental autoimmune encephalomyelitis via signaling through the aryl hydrocarbon receptor in dendritic cells

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UV-B irradiation is known to be one of the most important environmental stimuli acting on the skin. Interestingly, we have previously shown that irradiation with UVB light ameliorated experimental autoimmune encephalomyelitis (EAE) a well-known mouse model for human multiple sclerosis. These data suggested that besides genetic susceptibility factors, environmental stimuli taken up by the skin, such as UVB light, might play a role in the modulation of disease perpetuation. The transcription factor aryl hydrocarbon receptor (AhR) that is expressed in the skin and the central nervous system (CNS) can be activated by environmental factors including UV-B induced photoproducts of tryptophan. Thus, we investigated the role of AhR during the transmission of environmental stimuli, sensed in the skin and known to modulate CNS inflammation. Therefore, wild-type (wt) and AhR-deficient mice ($AhR^{-/-}$) were irradiated with UV B and immunized with myelin oligodendrocyte glycoprotein (MOG)- peptide. Whereas irradiated wt mice showed a delayed onset and reduced severity of disease, EAE perpetuation was comparable in UV-B-irradiated and non-irradiated $AhR^{-/-}$ mice. Flow cytometry and immunofluorescence staining revealed reduced numbers of pathogenic Th17 and increased levels of regulatory T cells (Treg) in the CNS from irradiated wt but not $AhR^{-/-}$ mice, indicating that AhR activation by UV-B might play a role during the expansion of Treg. Of note, the UV-B-induced activation of AhR was confirmed by the up-regulation of AhR target genes like cytochrome P450 family member A1 (CYP1A1) and CYP1B1. To characterize the underlying cellular and molecular mechanisms we deleted AhR in different subsets of dendritic cells (DC) that have been shown to mediate the expansion of Treg or directly in T cells. Interestingly, mice specifically lacking AhR in $CD11c^{+}$ mature DC or $CD207^{+}$ cutaneous DC UV-B-irradiation did neither ameliorate EAE perpetuation nor modulate Treg or Th17 numbers. Worth mentioning, that the deletion of AhR in neurons or directly in T cells had no impact on the UV-Binduced protection from EAE. Thus, our data indicate that AhR activation in DC might be required for transmitting the environmental factor UV-B, which is sensed in the skin into susceptible mice during MOG-induced EAE. Hence, these studies clearly demonstrate that the skin plays an important role in sensing environmental stimuli with impact on the development and progression of autoimmune disorders affecting other organs than the skin.

P137 | Reactive neutrophil responses dependent on the receptor tyrosine kinase c-MET limit cancer immunotherapy

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The receptor tyrosine kinase c-MET and its ligand HGF have emerged as critical drivers of resistance to oncogenic kinase inhibitors in various types of human cancers. Currently, c-Met inhibitors are used in the clinic to target oncogenic signaling in tumor cells. As HGF/c-MET signaling is also known to modulate immune responses, exploiting the immunoregulatory capacities could significantly broaden the clinical use of c-MET inhibitors.

Here, we report that concomitant c-MET inhibition promoted adoptive T cell transfer and checkpoint immunotherapies in relevant mouse tumor models independent of tumor cell-intrinsic c-MET dependence. Mechanistically, we found that in response to cytotoxic immunotherapies, HGF/c-MET signaling orchestrates a neutrophil response which limits the efficacy of anti-tumoral T cells. Neutrophils acquired immunosuppressive properties in tumor-draining lymph nodes and tumor tissues and suppressed tumor-specific T cells which had lower proliferative capacities and reduced effector functions. Concomitant METi impaired the reactive mobilization and recruitment of neutrophils into tumors and lymph nodes. Consequently, this promoted the expansion of anti-tumoral effector T cells and led to an enhanced tumor control. Importantly, high serum levels of HGF correlated with increasing neutrophil counts in cancer patients not responding to checkpoint blockade therapies.

In conclusion, our work reveals a role for the HGF/c-MET signaling pathway in neutrophil recruitment and function and suggests that concomitant c-MET inhibitor treatment may increase the efficacy of cancer immunotherapies in a variety of cancer patients.

P138 | Histamine modulates the production and secretion of RNase 7 in human keratinocytes

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Histamine and antimicrobial peptides (AMPs) are both important immunomodulatory mediators in the pathogenesis and maintenance of

inflammatory skin diseases like atopic dermatitis (AD) and psoriasis. RNase7, which belongs to the RNase family, is expressed constitutive in the upper layers of the epidermis and upregulated in lesions of AD and psoriasis. Since histamine is also elevated in inflammatory skin diseases, the aim of this study was to explore the impact of histamine on the production and release of RNase7 in human keratinocytes.

In monolayer cultures of human neonatal keratinocytes, we found that stimulation with histamine significantly enhanced RNase7 production at mRNA-level. To mimic the inflammatory conditions of Th1 and Th2 cytokine-mediated skin diseases, we stimulated keratinocytes with a combination of IFNy and IL-17 and with IL-4, respectively. While IL-4 reduced the production of RNase7, pre-incubation with histamine blocked the decrease. Stimulation with IFNy and IL-17 enhanced the production of RNase7, which could be further potentiated by pre-incubation with histamine. Additionally, we performed 3D skin equivalents, which are characterized by well differentiated keratinocytes. In this model IL-4 significantly increased RNase 7 production at mRNA level, which is in contrast to the data obtained in monolayer cultures. However, the increase of RNase 7 production could be validated on protein level by immunohistochemical staining. Histamine inhibited the increase of RNase 7 production in the 3D skin model. Interestingly, secreted RNase 7 protein, measured in the supernatants by means of Elisa, was elevated in the histamine-stimulated samples, while IL-4 did not show an impact of RNase 7 secretion. Thus, it seems that IL-4 and histamine differentially modulate RNase 7 production and release. As expected, IFNy increased the RNase 7 protein secretion in the 3D skin model, which could be again further potentiated by histamine.

Further experiments are necessary to elucidate the mechanism of histamine mediated RNase 7 production and release and to examine which histamine receptor subtype is involved in this process. However, these first results already indicate a possible role for histamine in modulating the impact of RNase7 in inflammatory skin diseases.

P139 | Epidermal cornification is associated with the expression of a keratinocyte specific set of pyroptosis-related genes

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The homeostasis of the epidermis depends on the continuous turnover of differentiating keratinocytes. The ultimate step of terminal differentiation is cornification, a mode of programmed cell death. In contrast to proinflammatory cell death modalities such as pyroptosis, epidermal cornification does not elicit inflammation. Therefore, we hypothesized that pyroptosis might be suppressed in cornifying keratinocytes. The expression of gene families implicated in pyroptosis was determined by RT-PCR, western blot and immunohistochemistry in keratinocytes undergoing differentiation in vitro and in human epidermis. Among the gasdermins (GSDMs), which induce cell death by pore formation in membranes, the expression of the classical pyroptosis mediator GSDMD was downregulated whereas GSDMA was significantly upregulated during keratinocyte differentiation. The expression of pyroptotic caspases was unaltered whereas caspase-14 was increased in differentiated keratinocytes. Remarkably, three confirmed negative regulators of inflammasome formation and pyroptosis, i.e. caspase recruitment domain 18 (CARD18), NLR family pyrin domain containing 10 (NLRP10), and pyrin domain containing 1 (PYDC1) were strongly induced during keratinocyte differentiation. Expression of GSDMA and CARD18 was also demonstrated at the protein level in differentiated keratinocytes in vitro and in vivo. Comparative transcriptomics of human tissues suggested that GSDMA, CASP14, CARD18, NLRP10, and PYDC1 are exclusively expressed in the skin. Together, our results suggest that human epidermal cornification is accompanied by a tight control of pyroptosis and warrant further studies of potential defects in the balance between cornification and pyroptosis in skin diseases.

P140 (OP02/03) | A novel mouse model for pemphigus vulgaris

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Pemphigus vulgaris (PV) represents a severe and difficult-to-treat blistering autoimmune disease of the skin and mucous membranes, despite recent therapeutic developments. IgG autoantibodies (autoab) mainly against desmoglein3 (Dsg3) induce loss of keratinocyte adhesion and lead to the clinical presentation of flaccid blisters and widespread erosions. As in other autoimmune disorders, mouse models have been very important in revealing crucial steps in the pathogenesis of PV. Our group has established a human leukocyte antigen (HLA)-transgenic mouse model of PV; the mice are transgenic for the PV-associated HLA-DRB1*04:02-DQ8 haplotype, the human CD4 co-receptor and they lack functional endogenous mouse major histocompatibility complex (MHC) class II (I-Aß$^{-/-}$). We have recently shown, that immunization of HLA-transgenic mice with recombinant human Dsg3 protein induces a CD4^{+} T cell-dependent production of Dsg3-specific IgG antibodies that are pathogenic in vitro. Using a set of five immunodominant Dsg3 CD4^{+} T cell epitopes for immunization of HLA-transgenic animals leads to a humoral immune response to Dsg3 protein in a HLA-DRß1*04:02-restricted manner. A limitation of this mouse model is that the HLA-transgenic mice express murine Dsg3 and although mouse and human Dsg3 share a high degree of homology, the immunization induced anti- Dsg3-IgG antibodies demonstrate only a weak cross-reactivity with the murine protein. To overcome this obstacle and to develop a preclinical animal model that closer mimics the human situation in PV, we developed a novel mouse model expressing a humanized Dsg3 protein. Mice on a C57BL/B6 background express the extracellular domain of human Dsg3, while the transmembrane

region and the intracellular domain of Dsg3 remain murine. After receiving mice homozygous for humanized Dsg3, these animals were crossed with the established HLA-transgenic C57BL/6 line. Finally, we succeeded in establishing a novel mouse line that is humanized for both the Dsg3 antigen and the HLA-class II, CD4 compartment. These mice develop normally and do not demonstrate any spontaneous clinical phenotype. The skin and the oral mucosa of the mice do not show any structural alterations by histopathological analysis. The humanized Dsg3 protein is regularly expressed in the epidermis as shown by immunofluorescent staining using anti-Dsg3 monoclonal antibodies as well as Dsg3-reactive IgG auto-ab of PV patients. A first set of experiments shows that Dsg3-immunization of HLA-transgenic mice that are homozygous for humanized Dsg3, results in a clearly decreased IgG response to human Dsg3 compared to HLA transgenic animals carrying murine Dsg3 and mice that are heterozygous for human Dsg3, respectively. Moreover, Dsg3-specific B cells (marginal zone and T1 B cells) can be detected in spleen and lymph nodes of HLA-transgenic, humanized Dsg3 mice by flow cytometry. In addition, we noticed an increase in naturally occurring $CD4^{+}CD25^{high}FoxP3^{+}$ regulatory T cells in lymphoid organs of this novel mouse model compared with wild-type animals upon immunization with Dsg3. In summary, we have expanded the field of preclinical PV models by establishing a novel mouse line that is humanized for both the autoantigen Dsg3 and the HLA haplotype which is highly prevalent in PV patients. This unique model holds great promise to investigate initial steps of loss of tolerance to the autoantigen Dsg3 and to characterize crucial immunological mechanisms finally resulting in the production of pathogenic auto-ab in PV.

P141 | Regulation of anaphylatoxin C3a receptor expression on human M2 macrophages by stimulating the histamine H4 receptor and the IL-4 receptor

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Background: Complement activation culminates in a strong amplification of the immune response. The anaphylatoxin C3a triggers inflammation by binding to its specific G-protein coupled C3a receptor (C3aR). M2 macrophages express histamine H1 receptor (H1R), H2R and H4R but not H3R at mRNA level.

Objective: Since the number of C3aR expressed on the cell surface affects the response to C3a, we investigated the expression levels of C3aR on human M2 macrophages in allergic situations where high levels of the Th2 cytokine IL-4 and histamine are present in a local microenvironment.

Methods: We differentiated human monocytes in vitro into M2 macrophages. Expression of H4R was analyzed by quantitative PCR in response to IL-4 compared to unstimulated M2 macrophages derived from atopic dermatitis patients and from healthy controls.

Expression of C3aR was measured by quantitative PCR and by flow cytometry: (i) On macrophages which were differentiated in vitro in the presence of histamine or the H4R agonist ST-1006, (ii) on fully differentiated M2 macrophages stimulated with different histamine receptor agonists, (iii) on M2- or IL-4 activated M2a macrophages derived from atopic dermatitis patients compared to healthy controls.

Results: The H4R agonist ST-1006 down-regulated C3aR expression in monocyte derived M2 macrophages at mRNA level and on the cell surface. The down-regulation of C3aR by ST-1006 is of functional relevance, since by analyzing C3a-induced IL-6 mRNA expression, we observed a diminished response to C3a in ST-1006 treated M2 macrophages compared to untreated cells.

Importantly, H4R mRNA was constitutively higher expressed in M2 macrophages derived from atopic dermatitis patients as compared to healthy controls whereas C3aR showed equal mRNA expression levels in both groups. The H4R mRNA expression was 10 fold up-regulated in contrast C3aR mRNA expression was 12 fold down-regulated by IL-4 in M2 macrophages obtained from healthy controls.

Conclusion: These data suggest that down-regulation of C3aR expression by mediators present in allergic situations such as IL-4 or histamine has an anti-inflammatory impact by reducing the sensitivity to C3a-induced down-stream signaling and thereby contributing to the regulation of local inflammatory responses in the skin.

P142 | Topical MTX-GNPs reduce IMQ-induced inflammation in mice

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Methotrexate (MTX) is a widely used immunosuppressive agent for the treatment of several autoimmune and chronic-inflammatory conditions, such as rheumatoid arthritis (RA) and psoriasis. MTX is usually administered systemically, which can cause side effects, including liver damage and kidney failure. Due to its polarity and high molecular weight topical MTX penetrates very poorly through the skin barrier and therefore needs a targeted drug delivery systems (TDDS) to overcome biological barriers. Gold nanoparticles (GNP) have been used as TDDS in cancer therapy and have recently been shown to deliver MTX into the skin by topical application (Bessar H et al., 2016).

Here, we explored the therapeutic potential of a novel topical MTX formulation in inflammatory skin disease. The skin permeability of MTX was increased via conjugation to GNP (MTX-GNP). Using the imiquimod (IMQ)-induced mouse model of psoriasis, where IMQ is applied on the ear of a mouse, we evaluated in vivo efficacy and functionality of MTX-GNPs.

Subcutaneous administration of MTX-GNPs ameliorated IMQ-induced inflammation in a dose-dependent manner, as measured by

ear thickness, erythema and scaling. The effect of systemic MTX-GNP compared to systemic MTX alone demonstrated a superior anti-inflammatory action on IMQ-induced inflammation. At day 5, mean ear thickness (MET) of MTX-GNP treatment group was 286.25 μm whereas the MET of MTX group was 335 μm, the former being significantly lower and latter not, compared to the treatment group who received IMQ only (MET=351 μm), *P* values being *P*=.0340 and *P*>.999, respectively. Additionally, MTX treatment displayed higher toxicity than MTX-GNP. Topical MTX-GNPs were formulated based on the systemic dose inducing the highest clinical efficacy but the least toxicity. Topical application of MTX-GNP gel significantly reduced IMQ-induced inflammation whereas a gel formulation of MTX, showed no improvement (MET of mice treated with IMQ only: 390 μm; MET of MTX-GNP group: 288 μm, MET of MTX group: 346.25 μm. MTXGNP vs. IMQ only: *P*=.0025; MTX vs. IMQ only *P*=.3767, all calculated on day 5).

GNPs significantly improve delivery of MTX to the skin, thus allowing transdermal application of the drug. Topical MTX-GNPs reduced IMQ-induced inflammation in mice without significant toxicity. MTX-GNPs should be considered as a non-steroidal therapeutic option for inflammatory skin diseases.

P143 | 4-n-nonylphenol switches non-regulatory T cells into a regulatory phenotype

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Recently we observed that injection of the arylhydrocarbon receptor (AhR)-agonist 4-n-nonylphenol (NP) inhibited the induction of contact hypersensitivity (CHS) in mice via induction of regulatory T cells (Treg). Although we identified dendritic cells as one of the major targets for NP in its immunosuppressive features, we were also interested to study whether NP may directly affect T cells. For this purpose, non-regulatory T cells ($CD4^+CD25$-) were isolated from trinitrochlorobenzene (TNCB)-sensitized mice and stimulated with NP or left untreated. After 48 hours cells were injected intravenously into naïve recipients which were sensitized 24 hours after injection. Intravenously injected Treg ($CD4^+CD25^+$) served as a control. Ear challenge with TNCB revealed that mice injected with NP-treated cells were significantly suppressed in their CHS response. This effect was almost identical to the Treg control. In contrast, injection of untreated $CD4^+CD25^-$ did not affect the sensitization in the recipients. For further characterization of the phenotype, cells used for injection were analyzed for the expression of the Treg markers Foxp3 and Garp. Flow cytometry analysis revealed a strong induction of both markers in response to NP, suggesting that NP is able to shift non-regulatory T cells into a regulatory phenotype. However, NP-Treg were not able to suppress the elicitation phase of CHS since the ear challenge response was not suppressed upon intravenous injection of NP-Treg into already sensitized mice. But when injected subcutaneously into the ears of sensitized mice before challenge the ear swelling response was significantly reduced. This indicates that NP-Treg upon intravenous injection do not migrate into the skin but into the lymph nodes. This may be due to the expression of specific tissue homing receptors which is currently under investigation. To prove whether NP induces Treg also in the human system $CD4^+CD25^-$ cells were isolated from peripheral blood mononuclear cells, incubated with NP and subjected to an in vitro suppression assay. NP-Treg suppressed the proliferative capacity of the responder cells, whereas unstimulated $CD4^+CD25^-$ did not. Together these data imply that NP exerts the capacity to switch non-regulatory T cells into a regulatory phenotype which is functionally suppressive.

P144 (OP06/02) | Obesity aggravates psoriatic cutaneous inflammation due to a selective enhancement of Th17/Th1 immune response

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The inflammatory skin disease psoriasis is often characterized by comorbidities with obesity as the most prevalent (with 30% of obese psoriasis patients in comparison to 16% obesity in the normal population). Epidemiological studies clearly associate obesity as an aggravating factor for skin inflammation in psoriasis but mechanisms mediating this are unknown. The aim of the present study was to investigate how obesity alters skin immune responses. To this end, a diet-induced obesity model was established by feeding of male C57Bl/6J mice with high-fat diet (HFD, 60% fat). To investigate the effect of obesity on Th17/Th1-mediated skin inflammation, a mouse model of 2,4,6-trinitrochlorobenzene (TNCB) contact hypersensitivity (CHS) was used. Challenging sensitized mice resulted in a significant increase of ear swelling of obese mice. Characterization of the immune response after the TNCB challenge revealed a significant elevation of IFNg- and IL-17-positive cells in draining lymph nodes (dLN) and an increase of IL-17, IL-21, IL-6, TNF in serum in comparison to the controls. Th2-dominated cytokine IL-4 was unchanged (serum) or decreased (dLN) due to HFD. Interestingly, the ear swelling of Th2-mediated CHS against fluorescein isothiocyanate (FITC) was strongly diminished in obese mice. This suggests a selective enhancement of Th17/Th1 and a decrease of Th2 immune responses by obesity.

Investigating the underlying mechanisms, we found an accumulation of $IL\text{-}17^+TNF^+$ cells in adipose tissue. In addition, the expression of Th1-polarizing cytokines IL-12 and IFNg and myeloid factors TNF, IL-6, iNOS and CCL2 were significantly increased. Interestingly, these factors (TNF, IL-6, iNOS, CCL2) and IL-17 were also significantly upregulated in the skin of obese mice, suggesting that obesity promotes immune cell infiltration both in adipose tissue and skin thereby leading to an aggravation of skin inflammation.

Next, we tested whether systemic factors, increased due to HFD, are responsible for alterations in cell phenotypes. Treatment of immune

cells with serum from obese mice resulted in an enhanced production of TNF by $Gr1^+$ cells and in elevated IL-17- producing $CD4^+$ and $CD8^+$ cells. The incubation of T cells with serum from obese mice during different polarization conditions led to a promotion of Th1 phenotype with a marked reduction of Tregs.

Taken together, our work indicates that obesity promotes skin inflammation by selective enhancement of Th17/Th1 immune response.

P145 | Identification of autoreactive B cell subpopulations in peripheral blood of pemphigus vulgaris patients

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Background: Pemphigus vulgaris (PV) is a paradigm of an autoantibody (auto-ab)-mediated autoimmune disease. Auto-ab mainly against desmoglein (Dsg)3 and Dsg1 have been shown to induce loss of keratinocyte adhesion in several in vitro and in vivo systems. In PV patients, these auto-ab cause painful blisters and erosions of the skin and mucous membranes. Thus, autoreactive B cells play a crucial role in the pathogenesis of PV and are currently in the focus of novel targeted therapies. So far, there are only few studies analysing auto-ab-producing B cells in PV patients. Therefore, we aimed at characterizing Dsg3-specific B cell subpopulations in PV patients at different stages of disease.

Methods: Dsg3-specific B cells were detected by flow cytometry using fluorescently labelled recombinant Dsg3 protein (Dsg3-AF647). To control for antigen-specific staining, we applied fluorescently labelled type VII collagen protein (COL7-AF647) that was expressed and purified in the same protein expression system. Dsg3-specific B cell subpopulations ($CD19^+CD27^-$ naïve B cells, $CD19^+CD27^+$ memory B cells and $CD19^+CD27^{hi}CD38^{hi}$ plasma cells) were analysed in peripheral blood of clinically well-defined PV patients (n=14) and age- and sex-matched healthy controls (HC; n=14). $CD19^+CD27^+Dsg3\text{-}AF647^+$ cells were sorted using flow cytometry and stimulated with R848/interleukin (IL)-2 for plasma cell differentiation. Dsg3-specific IgG-producing plasma cells were subsequently detected by enzyme linked immuno spot (ELISpot) assay.

Results: Dsg3-specific B cells were present in PV patients (0.11-0.53% of $CD19^+$ B cells) at higher frequencies compared to HC (0.09-0.22% of $CD19^+$ B cells). Using the control protein COL7-AF647, we noticed a very low staining comparable to the HC. When analysing the Dsg3-reactive B cell subsets, we detected higher frequencies of Dsg3-specific memory B cells in PV patients compared to HC, whereas Dsg3-specific naïve B cells and plasma cells, respectively, did not differ between PV patients and HC. In particular, Dsg3-specific memory B cells were present in PV patients in remission who were on minimal therapy whereas in patients receiving high-dose immunosuppressives, Dsg3-reactive B cells were markedly reduced. To demonstrate that Dsg3-reactive memory B cells secrete anti-Dsg3 IgG, cells were sorted and stimulated with R848/IL-2 in order to induce their differentiation into plasma cells in vitro. Sorted Dsg3-specific memory B cells from remitting PV patients produced anti- Dsg3 IgG in pronounced numbers while only very few cells produced IgG that bound to the control protein COL7. In contrast, $CD19^+CD27^+Dsg3^-AF647^+$ cells sorted from HC showed only a minor IgG-response to Dsg3.

Conclusion: Our results show that Dsg3-specific B cells are present at low frequencies in peripheral blood of PV patients. The presence of Dsg3-specific memory B cells in remitting PV patients, capable of producing anti-Dsg3 IgG upon in vitro stimulation, points towards a persistence of autoreactive B cell clones that may be responsible for causing a relapse during the progress of disease. The flow cytometry-based identification of Dsg3-specific B cells provides a very promising tool for a more precise immunomonitoring of PV patients in different stages of disease and finally it may serve as a helpful biomarker for therapeutic interventions.

P146 | Potential role of TGF-beta signaling in immune-checkpoint-therapy-induced resistance in malignant melanoma

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Metastatic melanoma is a highly aggressive malignancy with poor clinical outcome. Recently, significant breakthroughs have been achieved in the field of oncogenic signaling inhibition and in particular in checkpoint inhibition immunotherapy (anti-PD1, anti-CTLA-4) resulting in improved patient care, yet tumor regression often occurs, suggesting that tumor cells acquire therapy-induced resistance. In order to understand the underlying resistance mechanisms in malignant melanoma, we firstly used the Cancer Genome Atlas (TCGA) database. Increased expression of the immune suppressive Programmed death-ligand 1 (PD-L1) correlated with increased expression of TGF-beta. In accordance, ~40% of our analyzed melanoma cell lines induce PD-L1 expression in response to recombinant TGF-beta on mRNA and protein level. Moreover, all of our analyzed cell lines showed increased expression of additional immune suppressive factors, including trombospondin-1 (TSP-1), CCL-2 and TGF-beta1, the latter one is pointing to a positive "feed forward Loop", thus indicating an important role of TGF-beta pathway to initiate and sustain the adverse inflammatory response. Besides its well described roles in blocking immune cell function, TGF-beta is also linked to a process called epithelial-mesenchymal transition (EMT). Consequently we analyzed the expression of PD-L1 in response to the EMT-initiating transcription factor Zeb-1. In

contrast to recombinant TGFbeta, overexpression of Zeb-1 failed to induce PD-L1 expression thus we conclude that the suppression of immune cells by PD-L1/PD-1 ligation does not require a mesenchymal transition of melanoma cells. In line with this, we failed to relate high PD-L1 expression to BRAF V600E melanoma cell lines which are characterized by high endogenous Zeb-1 levels. In summary our data indicate that the activation of the TGF-beta pathway, supposedly activated by the release of TGF-beta from immunetherapy recruited infiltrating cytotoxic T cells, induced an immune suppressive tumor microenvironment which in turn favors tumor progression. TGF-beta blockade may therefore be a rational strategy to improve response rates in melanoma patients.

P147 | Glutathione deficiency in cystine/glutamate antiporter knockout mice is not sufficient for induction of dimethyl fumarate-mediated immune deviation

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Dimethyl fumarate (DMF) is a clinically approved drug that improves multiple sclerosis (MS) as well as psoriasis. In mice, DMF protects from experimental autoimmune encephalomyelitis (EAE), a murine model for T-cell-mediated autoimmune diseases. Those diseases have been shown to be associated with an aberrant induction of interleukin (IL-) 12 and IL-23-producing type I dendritic cells (DC), resulting in pathogenic Th1 and Th17 cell responses. We have previously shown that the treatment with DMF leads to the induction of HO-1 and the inhibition of STAT1 phosphorylation. This results in IL-10-producing and IL-23 and IL-12-suppressing type II dendritic cells that promote Th2 cells instead of pro-inflammatory Th1/Th17 cells. It is thought that this immune deviation results from the induction of oxidative stress, as DMF treatment leads to a depletion of the reduced form of intracellular glutathione (GSH), the cells' most important scavenger of reactive oxygen species (ROS). To specifically analyze the redox-mediated modulation of dendritic cell and T cell differentiation without other possible interfering effects of DMF, we isolated bone marrow derived dendritic cells (BMDC) from cystine/glutamate antiporter knockout (xCT.KO) mice. This genetic knockout of the cystine/glutamate antiporter results in diminished levels of glutathione, as cystine, an essential molecule for GSH synthesis, cannot be transported into the cell. First results showed that BMDCs isolated from xCT.KO mice have substantially decreased levels of glutathione when compared to wildtype cells. Furthermore, this inhibition of GSH synthesis results in an increase of reactive oxygen species. In contrast to our hypothesis, these alterations in redox homeostasis did not interfere with downstream signaling pathways known to be affected by DMF treatment, such as the induction of HO-1 or inhibition of STAT1 phosphorylation, as well as the subsequent inhibition of the proinflammatory cytokines IL-23 and IL-12. Most importantly, additional DMF treatment still induced HO-1 and suppressed STAT1 signaling in these GSH-depleted xCT.KO cells. Thus, GSH depletion alone is not sufficient to transform dendritic cells to a DC type II phenotype. Instead, additional intracellular targets of DMF seem to play an important role in the shift to an anti-inflammatory Th2 cell-dominated immune response. Alternatively, the genetic insufficiency of GSH might be compensated by other anti-oxidative pathways which we are currently investigating.

P148 | Cloning and analysis of bullous pemphigoid anti-BP180 IgG and IgE autoantibodies indicates predominance of VL1-47 gene usage and direct (from IgM) and indirect (through IgG) class switching to IgE

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In bullous pemphigoid (BP) severity of disease is correlated to IgG autoantibodies against the BP180 NC16A domain that cause subepidermal blisters both directly and by complement fixation. However, IgE autoantibodies are also detected in these patients, and are thought to contribute to pathology through activation of mast cells. Further supporting the importance of IgE in BP, omalizumab (anti-IgE) is therapeutic for some patients. We analyzed the autoantibody repertoire in two patients with BP to gain insight into how IgE develops and to determine if there might be common features among autoantibodies.

We first analyzed the IgG and IgE repertoire by antibody phage display (APD) from an active BP patient in whom we detected both IgG and IgE BP180-NC16 by ELISA. High-throughput screening of separate IgG and IgE phage libraries on a BP180-NC16A substrate identified 17 unique antibody clones (as defined by heavy chaincomplementarity region (H-CDR) 3 amino acid sequences and shared VJ gene usage). All clones were confirmed by positive NC16A phage ELISA and production of soluble single-chain variable fragments that bound the basement membrane by indirect immunofluorescence.

Of these 17 B-cell clones, 8 clones were only found in the IgG library, whereas 8 were only in the IgE library; one was detected in both isotype libraries. This one clone shows that at least some BP IgE is derived from class switch from IgG. Analysis of somatic mutations of variable heavy chains of 26 antibodies (abs) derived from these 17 clones (n=15 for IgE, n=11 for IgG) revealed significantly lower mutation counts in IgE variable heavy chain gene (VH) regions when compared to IgG VH gene regions (111.4 vs. 15.2730.85; meanSEM; P=.025), suggesting many IgE abs come from naïve B cells. Three IgE clones had members with <4 somatic mutations (compared to no IgG clone with <12 somatic mutations), also suggesting there is direct

class switching to IgE from IgM. On the other hand, the one IgE clone definitely derived from IgG class switch had higher mutation rates in its members (15.252) consistent with indirect class switching. These findings suggest BP IgE is derived both from direct switch from IgM and from indirect switch through IgG.

Analysis of light gene usage in this patient indicated the surprising finding of a predominant usage of a particular light chain gene, VL1-47, which paired with many clonally different heavy chains in both IgG and IgE anti-BP180-NC16A monoclonal abs. To rule out this was an artifactual pairing in APD and to determine if VL1-47 was predominant in another patient, we used tandem mass spectrometry to identify light chain usage in autoantibodies affinity purified on NC16A. This analysis showed marked enrichment (over 7x) of VL1-47 in NC16A-autoantibodies compared to all abs. The importance of the light chain in BP autoantibodies is in marked contrast to the usual importance of the heavy chain in most autoantibody-mediated diseases, e.g., pemphigus.

These data show two pathways leading to pathogenesis of BP IgE abs; one directly from naïve B cells and one from antigen-specific B cells. Additionally, the finding of a dominant light chain gene usage may provide a target for therapy in some of these patients. Finally, the multiple monoclonal, monovalent anti-BP-NC16A abs we isolated should be useful as reagents to identify the hemidesmosome in the epidermal basement membrane and as tools to dissect the pathophysiology of autoantibodies in the disease.

P149 (OP04/01) | The relevance of the C5a/C5aR1 axis for induction of auto-antibody response in Epidermolysis bullosa acquisita

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Background: Epidermolysis bullosa acquisita (EBA) is a blistering skin disease caused by autoantibodies (auto-Abs) against collagen type VII (Col7), one of the major components of anchoring fibrils responsible for adhesion of the dermal to the epidermal layer in skin. IgG auto-Abs recognize and interact with a part of murine Col7, which shows sequence homology to the von-Willebrand-factor A like domain 2 (vWFA2). Previously, we found that in an antibody-transfer induced model of EBA, C5a receptor 1 (C5aR1) deficiency protects the mice from disease development. This demonstrates the critical role for C5aR1 in the effector phase.

Furthermore, we have also shown the importance of the regulatory cross-talk between C5aR1 and Fcγ-receptors (FcγR), which are importantly involved in translating antibody-reactions into cellular responses. Previously, it was reported that EBA is mainly FcγRIV-dependent.

Material & Methods: For our experiments, we actively immunized EBA-susceptible (BL6/S) and nonsusceptible (BL6/J) wildtype (wt) mice, BL6/S C5aR1$^{-/-}$ and FcγRIIB$^{-/-}$ mice with vWFA2. The clinical phenotype was scored and blood samples were taken biweekly for 8 weeks. Col7 specific IgG serum auto-Abs were determined by ELISA and used for Col7 epitope mapping and ROS analysis. Finally, we assessed IgG Fc glycosylation profile, which defines pro- or anti-inflammatory properties of IgG antibodies. After 8 weeks, the spleen and draining lymph nodes were harvested for phenotypic and functional cellular analyses.

Results & Conclusions: We observed first clinical symptoms after 4-6 weeks in wt mice, whereas C5aR1$^{-/-}$ mice on susceptible BL6/S background were completely protected from EBA in the immunization-induced model.

The glyco-analysis of C5aR1$^{-/-}$ showed significantly decreased levels of proinflammatory agalactosylated auto-Abs and significantly increased levels of antiinflammatory highly galactosylated IgG1 in C5aR1$^{-/-}$ sera comparable to the nonsusceptible BL6/J sera, whereas BL6/S showed a reverse galactosylation pattern. Interestingly, also the epitopes recognized by auto-Abs derived from C5aR1$^{-/-}$ mice differed clearly from wt BL6/S mice. The ROS-assay results showed that activation of neutrophils with auto-Abs from EBA mice in the immunization induced model is also dependent on FcγRIII, whereas the rabbit-to-mouse Ab transfer model is only FcγRIV dependent.

Our findings identify the C5a/C5aR1 axis as an essential driver of EBA. Mechanistically, it is required for the induction of pathogenic auto-Ab production and Fc glycosylation towards a pro-inflammatory Ab type. Moreover, we showed for the first time the relevance of FcγRIII in this disease.

P150 | Altered levels of B cell subpopulations and immunoglobulins in patients with psoriasis

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Background: Psoriasis is a chronic inflammatory disease mainly driven by T lymphocytes that is exemplified by commonly used and emerging therapeutic strategies inhibiting proinflammatory cytokines secreted by T cells. However, B cell subsets have thus far largely been neglected in the pathogenesis of psoriasis.

Objective: To evaluate B cell subsets and levels of immunoglobulins in peripheral blood of patients with moderate-to-severe psoriasis before (T0) and upon successful treatment (T1).

Patients & Methods: Peripheral blood B cell subpopulations and immunoglobulins (Ig) from 31 patients with moderate-to-severe plaque psoriasis and 31 matched healthy controls were analyzed. Levels of total B cells, transitional B cells (trB), naïve mature B cells (NM), memory B cells (M), total plasma cells (PC), long-lived PC and plasmablasts (PB) were determined by flow cytometry. Cytometric

bead assays were used to measure serum levels of IgM, IgG, IgA and IgE.

Results: We show that B cell subsets in peripheral blood from patients with psoriasis differ from those observed in healthy controls. Frequencies and absolute numbers of B cells in total were moderately but not significantly decreased in patients with psoriasis. In-depth analysis revealed a significant elevation of trB, a moderate elevation of NM and a pronounced decrease of M. Total PC were significantly lower in affected patients. However, further differentiation showed a significant increase of short-lived high-frequency antibody-producing PB within the PC pool, which was accompanied by elevated serum levels of IgA and IgG. Longitudinal blood analysis after initiation of successful treatment, defined as at least 75% improvement of PASI, demonstrated a convergence of B cell subsets to those observed in controls. In contrast, IgA and IgG levels did not change markedly despite amelioration of skin inflammation.

Conclusions: Our findings may hint at a potential role of B cells in ongoing immunologic processes in psoriasis due to altered B cell subpopulations and immunoglobulin levels in peripheral blood of patients with severely inflamed psoriatic skin. Elevated IgG and IgA levels in combination with elevated PB support the concept of psoriasis as an autoimmune disease. However, the potential specific antigen(s) remain(s) to be elucidated.

P151 | Ineffective antibody-dependent cellular cytotoxicity in patients with cutaneous T cell lymphoma

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Mycosis fungoides is the most common type of cutaneous T cell lymphoma (CTCL) and is associated with bad prognosis in advanced stage of the disease. Targeted therapies and immune modulators are currently changing our understanding for the treatment of solid tumors, and CTCL as well. Natural killer (NK) cell-mediated antibody-dependent cellular cytotoxicity (ADCC) often being presumed to be a key mode of action of targeted therapies. In this study, we identified MHC I overexpressed on MF skin T cells by using single cell RNA sequencing and confirmed the overexpression by flow cytometrical analysis. The overexpression of MHC I acts as immune checkpoint suppressing NK-cell activity and NK-cell-mediated ADCC. In combination with MHC I blockade, NK cells induce enhanced ADCC activity against MF skin T cells. Understanding of the immunological mechanisms behind it will help improve NK cell activity in CTCL patients and overcome resistance to treatment.

P152 | Immunoregulation by fibroblast during dermal wound healing

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The wound healing process can be divided into three phases which do not occur in series but overlap. These predicted phases are inflammation, proliferation and maturation. As other complex processes wound healing is susceptible to interruption or failure leading to non-healing chronic wounds. A crucial checkpoint in the healing process is the resolution of inflammation which is regulated by M1/M2 macrophage (Ma) activity. The polarization from an inflammatory activated phenotype (M1) to an alternative pro-repair/resolving phenotype (M2) is regulated by the surrounding microenvironment. Several soluble factors, which inhibit the inflammatory activation of M1 or support the polarization toward M2, have been described including IL-10, transforming growth factor ß (TGFß), tumor necrosis factor-inducible gene 6 protein (TSG-6), or products of cyclooxygenase-2 (cox-2) such as prostaglandin E2 (PGE2) or D2 (PGD2). Despite that, the mechanisms that control this M1/M2 switch are not completely understood. Here we show that dermal fibroblasts (dFb), as it is known for mesenchymal stem cell, secrete under the influence of an inflammatory environment TSG-6 and cox-2 products. Underlining these facts we could furthermore show that the injection of dFb into mice with thioglycollate induced peritonitis promotes the activation of alternative macrophages releasing high amounts of IL-10. Consequently, administration of dFb in wound margins improves defective tissue repair in db/db mice by reducing inflammation and favoring prorepair macrophages. Based on these results we currently investigate whether dFb perform crucial immunoregulatory functions during dermal wound healing and further characterize the mechanisms how dFb induce the macrophage polarization.

To identify whether immunomodulatory factors are expressed by dFb during the course of wound healing we first analyzed wound lysates using the full-thickness wound healing model in mice. Wounds were harvested at different time points post wounding (pw). Different cell types were sorted via FACS, RNA was extracted and gene expression levels were determined. We observed in the early phase of healing process (day 1 till day 3 pw) increased expression levels of TSG-6 in dFb fractions compared to other cell types, an additional peak of TSG-6 expression occurred in dFb fraction starting at day 10 pw. These data correspond to the fact that TSG-6 is known to have anti-inflammatory and tissue reparative properties. As a member of the hyaluronan-binding protein family it interacts with hyaluronan (HA) and is involved in extracellular matrix remodeling. There are data describing that HA-rich ECMs interact with cells through receptors like CD44, which is also expressed on Ma. So far there is no known receptor of TSG-6 but its recognition via CD44 has been suggested and thus might be a possible

mechanism how TSG-6 mediates its immunomodulatory effect on Ma. Since dFb synthesize both,TSG-6 and HA, we will further explore the role of HA and TSG-6 in the immunomodulatory crosstalk between dFb and Ma.

P153 | Drug repurposing as a successful principle to identify drugs that alleviate experimental epidermolysis bullosa acquisita (EBA)

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Epidermolysis bullosa acquisita (EBA) is a prototypic immunobullous disorder caused by autoantibodies directed against type VII collagen (COL7), which is the major component of the anchoring fibrils at the dermal epidermal junction. Treatment of EBA is difficult and more specifically relies on general immunosuppression. The molecular mechanisms of disease, required for the pathogenesis, are poorly understood limiting the clinical development of rational targeted therapies. Several studies indicated the pivotal role of neutrophils in both induction and effector phases of EBA; therefore, these cells are considered as potential therapeutic targets for treatment of EBA. Drug repurposing represents an alternative to drug discovery and exploits new molecular targets of a known drug for different medical indications. Here, we tested this hypothesis with screening of 1200 FDA-approved compounds for their suppression of generated reactive oxygen species (ROS) from immobilized immune complex (iIC)-activated human neutrophils. Among the tested compounds, thirty three compounds repressed ROS generation by more than 50%. This group of compounds were then investigated for their dose dependency and ROS scavenging activities. The cytotoxic drugs were also excluded from further study. Via complementary screening, six drugs were identified and subjected to the further elucidation of the clinical potential effect in an antibody transfer-induced mouse model of EBA. Four of these drugs indicated disease alleviating impact on this disease model, from which one drug was selected by its potency of effect on reduction of the disease severity. To identify the comparative transcriptional profiling, we performed RNA sequencing (RNA-seq) of skin tissues obtained from EBA mice treated with this drug and its vehicle. The result of our expression profiling would provide information about the genes of relevant immune cells affected by our drug which could be then considered as a therapeutic target for EBA treatment.

P154 (OP02/01) | Human liver- and skin-derived NK cells exhibit antigen-specific memory responses

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Until now, vaccine strategies have been exclusively focused on promoting effector activity by the two classical arms of the adaptive immune system, namely B and T cells. Mounting evidence points to an important role for a third lymphocyte subset, natural killer (NK) cells, in host resistance to infections. Murine liver-resident NK cells were discovered with long-lived memory of haptens and viral antigens. Despite phenotypic analysis of human liver NK cells, the existence and consequences of antigen-specific NK cell memory still needs to be proven.

We isolated human liver NK cells from individuals vaccinated against hepatitis A/B and characterized them phenotypically and functionally in killing assays against these viral antigens. Furthermore, we evaluated the distribution of NK cells in epicutaneous patch test reactions of nickel-sensitized patients and assessed their antigen-specific capacity to affect nickel-pulsed target cells.

In contrast to the peripheral blood, 2 distinct NK cell populations were found in the liver based on their expression of CD16 and CD49a. $CD49a^{+}CD16^{-}$ liver NK cells (54.6% 4.2 of total NK cells) performed antigen-specific killing comparable to CD8 T cells. Blood-derived and CD49a-$CD16^{+}$ liver NK cells did not exert antigen-specific cytotoxicity, but recognized MHC-Ilow target cells. Although absent in healthy human skin, 57.8 5.1% of total NK cells in nickel-induced epicutaneous patch tests were found to belong to the $CD49a^{+}CD16^{low}$ NK cell subset capable of lysing nickel pulsed target cells.

These results suggest that antigen-specific memory NK cells in humans can be found in the liver and inflamed skin, which might lead to novel strategies of vaccination by harnessing this NK cell subset.

P155 | Tissue factor-PAR2 signaling mediates inflammation in contact hypersensitivity

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Tissue factor (TF) has a mandatory function in hemostasis and thrombosis and protease-activated receptor-2 (PAR2) signaling is a strong mediator of immune processes in cancer and inflammation. However, their interaction with the innate and adaptive immune system in cutaneous inflammation is poorly understood.

PAR2 is a G protein-coupled receptor that is activated by a broad array of serine proteases including the TF ligands activated coagulation factors VII (FVIIa) and X (FXa) and the transmembrane peptidase matriptase.

Our study focused on TF-PAR2 signaling using contact hypersensitivity (CHS) as a model for the allergic contact dermatitis in humans. We analyzed the function of TF in wildtype mice using an anti-TF antibody. PAR2 cleavage by coagulation proteases was investigated by transgenic mouse strains abolishing FXa (PAR2 G37I), TFFVIIa- FXa (PAR2 R38E) or matriptase (PAR2 K36E) signaling. In addition, the cell specificity of PAR2 signaling was elucidated by PAR2 deletion in $CD11c^+$ dendritic cells (DC) and myeloid cells ($CD11c\text{-}cre^{+/-}/PAR2^{flox/flox}$ and $LysMcre^{+/-}/PAR2^{flox/flox}$ mice strains, respectively).

TF inhibition significantly reduced the cutaneous immune reaction, as demonstrated by an impaired ear swelling and an alleviated immune cell infiltration (histology, flow cytometry). TF blockade also strongly decreased the Tc1-mediated T cell response after hapten-specific restimulation in vitro, shown by an abrogated T cell proliferation and a diminished Tc1 cytokine production (IFN-gamma, IL-2). Phase-specific TF abrogation revealed a pro-inflammatory function of TF in both, the induction and effector phase of CHS.

Abolished PAR2 cleavage by the coagulation proteases FXa (PAR2 G37I) or TFFVIIa- FXa (PAR2 R38E) significantly reduced the CHS reaction at 8 and 24 hours after the challenge and displayed a reduction of the cutaneous immune cell infiltrate and the hapten-specific Tc1 response. In particular, abrogated TF-FVIIa-FXa mediated PAR2 cleavage (PAR2 R38E) resulted in a stronger inhibition of the allergic inflammatory reaction than the exclusive resistance to the proteolytic effect of FXa (PAR2 G37I). In accordance to these pro-inflammatory effects of specific PAR2 cleavage, lack of matriptase-mediated PAR2 signaling led to a significantly attenuated CHS reaction at the early effector phase of CHS.

Investigating the cell-specificity of PAR2 signaling in CHS uncovered a pivotal role of myeloid immune cells at the early stage of the allergic cutaneous inflammation. Absence of PAR2 expression in $LysM^+$ myeloid cells resulted in a significantly diminished CHS reaction at 4 and 8 hours after challenge as shown by a reduced ear swelling, cutaneous inflammatory infiltrate and hapten-specific Tc1 response. In contrast, PAR2 signaling in $CD11c^+$ DC did not influence the CHS reaction in any of these parameters.

TF-PAR2 signaling is a key mediator of inflammation in CHS and may provide novel targets in the topical and systemic treatment of cutaneous inflammatory diseases.

P156 | High-dose immunoglobulins induce a reduction of desmoglein 3-specific IgG antibodies in a human leukocyte antigen-transgenic mouse model of pemphigus vulgaris.

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Our group has established a human leukocyte antigen (HLA)-transgenic mouse model of pemphigus vulgaris (PV) using the HLA-haplotype (HLA-DRB1*04:02-DQ8) that is highly prevalent in PV patients. The mice are further transgenic for the human CD4 coreceptor and do not express functional endogenous mouse MHC class II ($IA\text{-}ß^{-/-}$). Immunization with recombinant human desmoglein 3 (Dsg3) protein leads to a HLA-DRB1*04:02-restricted activation of Dsg3-reactive $CD4^+$ T lymphocytes and subsequently to the production of pathogenic Dsg3-specific IgG antibodies (ab). High-dose immunoglobulins (IVIg) are successfully used in the treatment of mostly severe and recalcitrant PV leading to a reduction of circulating auto-ab. However, the mode of action of IVIg in PV is not completely understood, yet. Hence, the aim of this study was to investigate the immunological effects of IVIg treatment in the HLA-transgenic mouse model. HLA-transgenic mice (n=11) were immunized with recombinant human Dsg3 (day 0 and day 14) for the induction of a Dsg3-specific $CD4^+$ T cell and B cell response and treated with IVIg (2 g/kg body weight) once a week for a 4-week treatment period. Mice that received phosphate buffered saline (PBS; n=13) or methylprednisolone (MP; (20 mg/kg body weight; n=10) instead were used as controls. Blood was taken weekly for the analysis of circulating anti-Dsg3- IgG by human Dsg3 enzyme-linked immune assay (ELISA) and mice were sacrificed at different time points during treatment. T cell subsets, including naturally occurring regulatory T cells ($CD4^+CD25^+FoxP3^+$ Treg) and Dsg3-reactive interleukin (IL)-4- or interferon-gamma (IFN-γ)-producing T cells were analysed within spleen and lymph node cells by flow cytometry and enzyme linked immuno spot (ELISpot) assay, respectively. PBS-treated control mice demonstrate a robust anti-Dsg3-IgG response which is detectable after the second Dsg3-immunization (day 14). Mice treated with MP did not differ from the PBS controls in the humoral immune response to Dsg3. In contrast, about half of the IVIg-treated mice showed a strongly reduced anti- Dsg3-IgG response (5 of 11 mice). By flow cytometry, we did not detect a significant difference in the number of Treg cells in lymphoid tissues of PBS-, MP and IVIg treated animals, respectively. Dsg3-reactive IL-4- and IFN-γ- producing T cells were identified within spleen of Dsg3-immunized mice. However, the frequencies of these two Dsg3-reactive T cell subsets did not differ between the three treatment groups. Our study reveals an immunomodulatory effect of IVIg on the humoral immune response to Dsg3 in the HLA-transgenic mouse model of PV that so far is not linked to an alteration of Treg or major Dsg3-reactive T cell subsets. The HLA-transgenic mouse model provides an unique in vivo system to further investigate the IVIg induced effects in PV.

P157 | Elevated IL-31 serum levels in bullous pemphigoid patients correlate with eosinophil numbers and are associated with BP180-IgE

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Bullous pemphigoid (BP) is the most common autoimmune blistering disease. Immunopathologically BP is mediated by anti-BP180-IgG and anti-BP230- IgG autoantibodies, which are directed against structural proteins of the hemidesmosome. The clinical picture of BP is characterized by erythematous plaques and tense blisters accompanied by severe pruritus. One important mediator of pruritus and inflammation is Interleukin-31 (IL-31). Thus, we hypothesized that IL-31 is elevated in BP and plays a role in its pathogenesis.

Using ELISA we found elevated IL-31 levels in serum and blister fluid of symptomatic BP-patients. We correlated IL-31 serum levels with different activity markers of BP: the main autoantibodies anti-BP180-IgG and anti-BP230-IgG, IgE and eosinophils in serum and lesional skin. We found a positive correlation of IL-31 and the number of eosinophils in peripheral blood as well as in lesional skin. Moreover, BP180 IgE autoantibody serum titers, which are increased in the majority of BP patients, were measured by ELISA and showed a positive correlation with IL-31 serum levels. FACS analyses of BP blister fluid identified granulocytes as the main IL-31 producing cell population.

In conclusion we show a correlation of elevated IL-31 serum levels in symptomatic BP-patients with activity markers of disease and an association with a certain autoantibody panel. Moreover, our results indicate that eosinophils are responsible for the elevated IL-31 levels observed in BP, since elevated IL-31 serum levels are associated with eosinophilia in peripheral blood and lesional skin and granulocytes were identified as the main source of IL-31 in blister fluid. Our data suggest that IL-31 plays a functional role in the pathogenesis of BP and may reflect disease activity.

P158 (OP02/06) | Induction of T cell hyporesponsiveness by CD73-expressing dendritic cells is crucial for tolerization in contact hypersensitivity reactions.

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The ecto-5′-nucleotidase CD73 converts extracellular adenosine monophosphate, which is a product of ATP degradation by CD39, to adenosine (ADO). ADO has well established anti-inflammatory effects and dampens T cell activation. To assess the role of ADO production by DC during T cell activation we first analysed CD73 expression by bone marrow derived (BM) DC and found substantial expression of CD73 in immature (i.e. $MHCII^+$, $CD8^+$ $CD11c^+$) BMDC (40-50%) as compared to mature (LPS treated) BMDC (5-10%). For functional analysis, immature OVA peptide pulsed BMDC from CD73 deficient animals ($CD73^{-/-}$) and wildtype controls were co-cultured with OT-II T cells. Here comparable proliferation in all samples was observed. When BMDC-stimulated OT-II T cells were subsequently re-stimulated with anti-CD3/28, reduced proliferation was recorded in samples with OT-II T cells after incubation with control-BMDC. In contrast, $CD73^{-/-}$ BMDC failed to induce this hyporeactivity in OT-II T cells. Moreover, blocking of ADO receptors on OT-II T cells during culture with control BMDC abrogated the effect, whereas addition of the ADO agonist NECA to $CD73^{-/-}$ BMDC-OT-II T cell cultures re-established hyporeactivity. Therefore, we conclude that production of ADO by CD73 expressing DC is crucially involved in induction of hyporeactive T cells.

To investigate whether similar mechanism are operative in tolerizing conditions in vivo, we used a CHS-tolerance model whereby application of DNTB to the abdomen of mice renders them tolerant to subsequent sensitization and challenge with DNFB. Here we show that both, the tolerogen DNTB as well as the sensitizer DNFB induced similar migration pattern of the different DC subsets from skin to draining lymph nodes. However, after DNTB application in comparison to solvent or DNFB, substantially higher expression levels of CD73 was observed in all the different smDC subsets. Of note, $CD73^{-/-}$ animals were resistant to tolerization by DNTB. Moreover transfer of $MHCII^+$ skin cells from tolerized control mice, but not from $CD73^{-/-}$ mice, was able to confer tolerance. Thus, these data indicate a crucial role of $CD73^+$ skin DC in regulating tolerance to haptens.

When isolated T cells from “tolerized” (DNTB) wild type mice were restimulated with antigen-pulsed BMDC or anti-CD3/CD28, respectively, we found reduced proliferation of $CD4^+$ T cells, reduced production of IL-2, IFNγ, TNFα, IL-1β, upregulation of erg2, egr3, ccl1 and tnfrsf9 mRNA (all of which are indicators for anergic T cells) and increased frequency and proliferation of $CD4^+CD25^+FoxP3^+$ cells in wildtype mice as compared to $CD73^{-/-}$ animals. Thus, from these data we conclude that regulated expression of CD73 and production of ADO by skin DC is crucial for induction of a hyporesponsive (i.e. anergic) state of T cells that is involved in tolerance induction during CHS responses.

P159 | Lipocalin 2 plays a role in palmoplantar pustular psoriasis

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Palmoplantar pustular psoriasis (PPP) is a rare chronic immune-mediated skin disease of the palms and soles. Lesions are characterized by erupting, sterile intraepidermal pustules which are often located within psoriasis vulgaris (PV)-like erythematous and desquamating plaques. While PPP is frequently associated with extra-palmoplantar

PV lesions and a family history of PV, it is rather recalcitrant towards medications used for PV treatment. So far, the PPP pathogenesis is poorly understood and biomarkers for inflammatory activity are missing. This study aimed at the identification and characterization of key players in PPP. Corresponding to this objective, we first quantified levels of several blood mediators in 60 PPP patients as well as healthy participants. Lipocalin 2 (LCN2) emerged as being highly upregulated in PPP. Its levels were independent of age, gender, or concomitant PV. The analysis of skin biopsies demonstrated that keratinocytes were important LCN2 producers in PPP skin lesions. In vitro stimulation studies with isolated keratinocytes revealed LCN2 production upregulated by IL-1β This upregulation was further enhanced by IL-17 and TNF-α, while IL-22 had no effect. A functional relationship between IL-1β and LCN2 was also evident by the strong positive correlation between blood levels of these parameters in PPP patients. We then investigated the association of LCN2 blood levels with clinical parameters. Here, a positive correlation was found for PPP pustule score and DLQI, but not for disease duration. Furthermore, LCN2 blood levels showed a positive correlation with the levels of the pro-atherogenic molecule resistin. Based on these data, we draw the following conclusions: Systemic levels of LCN2, found to be elevated in PPP patients, seem to be derived from IL-1β-exposed lesional keratinocytes and may contribute to the recurrent skin neutrophil infiltration and pustule formation typical for this disease. At the same time, these data point to a role of IL-1β in PPP and an increased pro-atherosclerosis risk for affected patients.

P160 | Clinical improvement after treatment with anti-IgE antibody is associated with reduced frequencies of IFN-gamma-, IL-10- and IL-31-secreting T cells in patients with chronic spontaneous urticaria

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Introduction: Patients with chronic spontaneous urticaria (CSU) suffer from recurrent itching wheals with or without angioedema. The standard therapy for CSU patients is the use of antihistamines. For patients who remain symptomatic despite antihistamine treatment the anti-IgE antibody omalizumab has been proven efficacious. However, its mechanisms of action are still not fully understood and immunological data analysing responder versus non-responder patients are rare. To obtain deeper insights in the immunological changes induced by anti-IgE treatment, we analysed both receptor bound IgE on different peripheral blood cells and frequencies of different T cell subtypes during the course of the treatment in CSU patients.

Methods: CSU patients (n=15) received monthly subcutaneous injections of omalizumab for up to six months. CU-related symptoms were assessed by both the urticaria control test (UCT) and the CU quality of life score (CU-Q2oL). According to their changes in the UCT score, patients were subdivided into full- (n=6; FR), partial- (n=6; PR) and non-responders (n=3; NR). Peripheral blood was drawn prior to each injection determining the concentration-dependent reactivity of patients' basophils to specific anti-FcεRI and unspecific fMLP stimulation by basophil activation test. Furthermore, the impact of anti-IgE treatment on the amount of cell-bound IgE was analysed on FcεRI$^+$ (e.g. on monocytes, dendritic cells, basophils) and FcεRII$^+$ cells (e.g. on B cells, eosinophils) by flow cytometry, respectively. In addition, the frequencies of IFN-γ-, IL-5-, IL-10- and IL-31-secreting T cells were measured after in vitro expansion using anti-CD3/CD28 beads by ELISpot assay. The clinical response to anti-IgE-treatment was correlated with the different immunological parameters.

Results: All patients treated with omalizumab showed a decrease in surface IgE and FcεRI expression on basophils during the observation period of 6 months, while the responsiveness of basophils to stimulation with anti-FcεRI increased. Accordingly, the amount of cell-bound IgE on the remaining FcεRI$^+$ and on FcεRII$^+$ cells declined except for B cells. Here, surface IgE remained unchanged in the FR and PR group at the end of the treatment period, but increased in the NR population. Interestingly, we could also detect reduced frequencies of IFN-γ-, IL-10- and IL-31-secreting T cells after 6 months in FR and PR with the strongest reduction in the FR group, whereas the respective subpopulations increased in NR patients. In contrast, numbers of IL-5-producing cells were not affected by anti-IgE treatment. Correlating the clinical outcome with the immunological parameters revealed that an improvement in CUQ2oL and UCT was highly associated with a decrease in basophil bound IgE and a decline of IL-10- and IL-31-secreting cells.

Conclusions: In addition to the reduction of cell-bound IgE and down-regulation of FcεRI, our results show that treatment of CSU patients with anti-IgE antibody interferes with distinct T cell subsets. However, the role of IFN-γ-, IL-10- and IL-31- secreting T cells in the pathophysiology of CSU has to be further elucidated.

P161 | Increased levels of CCL2 in Schnitzler's Syndrome

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Schnitzler's syndrome (SchS) is a rare acquired autoinflammatory disease characterized by urticarial rash, bone and joint pain and fever and associated with monoclonal gammopathy. The pathophysiology

in SchS, which includes the overactivation of the IL-1 system, remains widely unknown.

Aiming at the identification of further pathogenetic players in SchS, we individually measured the levels of a range of parameters in the peripheral blood of afflicted patients. Healthy participants as well as patients with psoriasis and acne inversa (AI) were included as controls. In these investigations, CCL2, a chemoattractant for monocytic and further mononuclear immune cells, turned out to be one of the factors most significantly elevated compared to healthy participants. In contrast, no CCL2 increase was noticed in psoriasis and AI patients. CCL2 levels were independent of gender and age of SchS patients. The study of the association of CCL2 blood levels with clinical parameters revealed a positive correlation with disease activity, measured by physician global assessment (PGA), but not disease duration. Furthermore, CCL2 levels correlated with BMI and serum LDL cholesterol levels of SchS patients but were independent of other serum lipids, neutrophilia and IgM levels. In vitro stimulation assays demonstrated strong CCL2 production capacity of PBMC and fibroblasts, but not epithelial or endothelial cells. Investigating a range of inflammatory mediators, only IL-1β (PBMC, fibroblasts) and TNF-α (fibroblasts only) turned out to be important CCL2 inducers in these cells. TNF-α and IL-17 further strengthened the CCL2-inducing effect of IL-1β in fibroblasts. In line with the in vitro data, in SchS patients a strong and moderate positive correlation of CCL2 levels with the serum levels TNF-α and IL-1β, respectively, was found. Therapeutic IL-1β blockade decreased CCL2 levels in SchS patients after already 1 week.

These data suggest that CCL2 is a component of the inflammatory cascade in SchS and may be derived from IL-1β- and TNF-α-exposed fibroblasts and immune cells. Elevated systemic CCL2 levels may be used as a marker of disease activity and may point to metabolic alterations in these patients.

P162 | Phosphodiesterase-4 inhibition alleviates psoriatic skin phenotype of K5.Stat3C mice

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Apremilast is a novel, orally deliverable small molecule that specifically targets phosphodiesterase-4 (PDE4) and therefore modulates the expression of a network of proinflammatory and anti-inflammatory mediators implicated in psoriasis and psoriasis arthritis. It is being studied in multiple Phase III clinical trials and has been shown to reduce the severity of moderate to severe plaque psoriasis, as well as improving difficult-to-treat nail, scalp and palmoplantar psoriasis. Currently it is used for the treatment of patients with moderate to severe plaque psoriasis. Even though the efficacy of Apremilast has been clinically demonstrated, little is known about the mechanism by which Apremilast modulates the immune system in psoriasis. We investigated the effect of Apremilast on the cytokine profile in a mouse model of psoriasis. K5.Stat3C transgenic mice express a constitutively active Stat3 (Stat3C) under the control of the keratin 5 (K5) promoter to enable its expression in the basal epidermal layer. These mice rapidly show upregulation of several genes and proteins associated to the pathogenesis of psoriasis, and skin wounding such as tape-stripping is associated with the development of skin lesions macroscopically and histologically identical to those found in psoriatic patients (Koebner phenomenon).

After psoriatic lesions development, K5.Stat3C transgenic mice were either left untreated or treated with 2 or 6 mg/kg Apremilast per day for 2 weeks. K5.Stat3C transgenic mice treated with 6 mg/kg/day Apremilast showed a reduction of the severity of psoriatic lesions whereas treatment with 2 mg/kg/day led only to moderate improvement. The reduction of severity was accompanied with a dramatically decreased epidermal thickening and a reduced immune cell infiltrate. Moreover, inflammatory cytokine levels including IL-1beta, IL-18, IL-17A and IL-22 were significantly reduced in lesional skin of K5.Stat3C transgenic mice treated with Apremilast at 6 mg/kg/day as compared to vehicle-treated mice.

Collectively, we showed that Apremilast significantly reduced the severity of psoriatic lesions in K5.Stat3C transgenic mice through the reduction of pro-inflammatory cytokines known to be involved in psoriasis.

P163 | Enhanced IL-9 production by skin T cells using TH9 promoting cytokines

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TH9 cells are identified by the production of IL-9 and play a role in the pathogenesis of inflammatory skin diseases. We investigated the effects of IL-9 skewing cytokines on skin resident T cells because better understanding of their biology may contribute to the development of novel therapeutic approaches.

Healthy human skin biopsies were cultured on cell foam matrices (grids) in the presence of either IL-2 and IL-15 (standard condition) or IL-2, IL-4 and TGF-beta (TH9-promoting condition).

Both culture conditions favored the proliferation of $CD45RO^+$ T cells. TH9-promoting conditions yielded significantly higher numbers of $CD4^+$ T cells as compared to standard conditions, while the frequency of T cells expressing the proliferation marker Ki-67 was similar under both conditions after 4 weeks. A differential expression of the skin-resident T cell markers CD69, CD103, and the skin homing receptors CLA and CCR4 was found on T cells using either culture condition. Paired analysis of T cells freshly isolated from skin and after 4 weeks of culture revealed drastic and significant downregulation of CD69 under both culture conditions and a trend towards upregulation of

CD103 under TH9-promoting conditions. IL-4 and TGF-beta synergistically downregulated CD69 expression. Significantly more T cells produced IL-9 under TH9-promoting conditions as compared to standard conditions upon CD3/CD28 stimulation and PMA/ionomycin restimulation. $CD3^+CD56^+$ cells were identified under standard conditions only.

We show for the first time that IL-2, IL-4 and TGF-beta promote the expansion of IL-9-producing T cells from healthy human skin, thus enabling further studies about their functional role in the skin.

P164 | FlgHrnr-deficient mice on C57BL/6 and Balb/c background exhibit phenotypic differences in a model of experimental atopic march, thus reflecting genetic variances in humans

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The concept of the atopic march describes the progression of atopic disorders from atopic dermatitis (AD) to allergic rhinitis and asthma. The risk to develop atopic diseases is complex and strongly influenced by both genetic and environmental factors. Filaggrin (Flg) and hornerin (Hrnr) are both key components during the terminal differentiation of the stratum corneum in human skin and loss-of-function mutations in these skin barrier proteins are a known major risk for AD; and moreover, for the further development of asthma. Previously, we provided experimental evidence that the barrier-disrupted $FlgHrnr^{-/-}$ mice on C57BL/6 mice are susceptible to acute AD-like dermatitis and the further proceeding of the atopic march.

Since clinical manifestations vary among individuals and genetic background in mice is responsible for various degrees of inflammation, such as seen in humans with different atopic status, we were interested to extend our study in mice at Balb/c background. Therefore, we backcrossed C57BL/6 $FlgHrnr^{-/-}$ mice systematically to Balb/c mice for 10 generations.

In our experimental atopic march model, mice were treated topically with MC903 (calcipotriol; a low-calcemic analogue of vitamin D3) which triggers an AD-like phenotype by inducing thymic stromal lymphopoietin (TSLP) in keratinocytes. Application was done during epicutaneous sensitization with OVA together with DBP (Dibutylphthalat) and mice were subsequently exposed to an OVA-aerosol challenge to induce asthma-like features.

As shown for $FlgHrnr^{-/-}$ mice on C57BL/6 background, we found that Balb/c $FlgHrnr^{-/-}$ mice treated with MC903 showed a worsened AD-like phenotype compared to Balb/c wildtype mice. Concurrently, percutaneous antigen sensitization was facilitated in MC903-treated $FlgHrnr^{-/-}$ mice as they showed an increased production of Th2-like immunoglobulins, such as total IgE and OVA-specific IgG1. When measuring asthma-like features, we found that MC903-treated $FlgHrnr^{-/-}$ mice showed an aggravated experimental allergic asthma with an increased total cell count in bronchoalveolar lavage (BAL), a higher portion of inflammatory infiltrates (mainly eosinophils) into the lung and slightly increased Th2 cytokines in the BAL fluid.

When compared to C57BL/6 mice, Balb/c mice showed a clear Th2-deviated immune phenotype as they had clearly increased levels of IgE and OVA-IgG1, which further influences the outcome of asthma-like features. Most interestingly, the magnitude of exacerbated clinical symptoms after MC903-treatment was even more prominent in $FlgHrnr^{-/-}$ mice on Balb/c background compared to C57BL/6 $FlgHrnr^{-/-}$ mice.

Undoubtedly, genetic background in C57BL/6 and Balb/c mice is responsible for Th1- or Th2-deviated immune responses, with Balb/c mice representing a hyperresponsive Th2-prone strain compared to C57BL/6, which exhibit a Th1- dominated response. Accordingly, our model perfectly reflects the genetic variance in patients. Thus, $FlgHrnr^{-/-}$ mice on the Balb/c background perfectly depict a model to further study how skin barrier impairment triggers cutaneous immune response and the development of allergic respiratory inflammation.

P165 | Effects of anthralin on innate factors and cytokeratins expression by keratinocytes

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Psoriasis is a Th17-meidated chronic inflammatory skin disease with aberrant keratinocyte proliferation and differentiation. Th17 cytokines like IL-17, IL-22 and IL-23 are critically involved in psoriasis pathogenesis that results in epidermal thickening and production of innate factors such as antimicrobial peptides. Topical treatment of psoriasis with anthralin is highly effective although the exact mode of action of this drug in psoriasis is not fully understood. We aimed to study the direct effects of anthralin on keratinocyte proliferation, differentiation and production of innate factors like antimicrobial factors. To test the effects of anthralin we used (i) primary keratinocytes, (ii) a 3D psoriasis tissue models and (ii) skin biopsies from patients treated with anthralin. In addition we studied the effects of anthralin in monolayer and multilayer keratinocyte cultures stimulated with IL-17A and IL-22. Anthralin directly induced cell apoptosis in vitro in monolayer cultures but not in multilayer cultures treated with IL-17A and IL-22. Yet, keratinocyte proliferation was impaired by anthralin in vitro and in vivo. In lesional skin anthralin rapidly normalized cytokeratin (CK)16 expression. Moreover, anthralin suppressed DEFB4 expression in vitro and in vivo, while other antimicrobial peptides and innate cytokines studied like IL-6 and IL-8 were regulated differently in vitro and in vivo. Taken together anthralin

has direct effects on keratinocytes beyond the impairment of proliferation. CK16 and DEFB4 expression by keratinocytes were both suppressed by anthralin.

P166 | Epicutaneous allergen exposure induces pathogenic IL-22-producing T helper cells

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Atopic dermatitis (AD) is a common inflammatory skin disease resulting from epidermal barrier dysfunction and an immune response dominated by IL-4-producing Th2 cells and IL-22-producing Th22 cells. Environmental antigens like food allergens are thought to influence the clinical course of AD. We aimed to study the pathogenic phenotype of food allergen-specific T cells activated through the skin or by oral exposure and their potency to transfer skin inflammation. Ovalbumin (OVA) TCR transgenic mice were sensitized epicutaneously with OVA or were fed OVA. $CD4^+$ T cells from skin or mesenteric lymph nodes were phenotyped for cytokine expression and transferred into naïve BALB/c mice. Recipient mice were challenged with OVA epicutaneously. Skin inflammation was determined histologically. T cells activated through epicutaneous or oral OVA exposure in donor mice both migrate to skin lymph nodes after adoptive transfer and epicutaneous OVA exposure of recipient mice. Importantly, AD-like skin inflammation could only be induced by the transfer of epicutaneously primed OVA T cells. Analysis of the immune phenotype demonstrated an IL-22/IL-17A-dominated immune phenotype of skin-pathogenic T cells. IL-22 seems to be the critical cytokine for the development of AD and is induced in this model by epicutaneous sensitization with OVA.

INFECTIOUS DISEASES

P167 | Comparison of antimicrobial efficacy of a plasma jet and a DBD plasma source in vitro

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Aim: Cold atmospheric pressure plasmas are mostly used for decontamination and sterilization of implants and heat-sensitive medical products. However, the direct use on the patient is conceivable as more and more about the complex interactions between plasma, micro-organisms and human tissue is understood. This study investigates the antimicrobial efficacy of a pulsed, cold atmospheric pressure plasma jet on micro-organisms causing skin infections, such as Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, and Malassezia pachydermatis and compares its activity to a DBD plasma source based on LTCC (low temperature cofired ceramic) technology.

Methods: Micro-organisms were plated onto MH2 agar plates in accordance to DIN 58940-3. Plasma treatment was performed using the plasma-BLASTER jet (Tigres, Marschacht; provided by INNOVENT Jena e.V.) and with the DBD plasma source (provided by Technische Universität Ilmenau, Institut für Mikro- und Nanotechnologien). Air was used as process gas and treatment times were varied. After treatment, MH2 plates were incubated at 37°C for 24 hours under aerobic conditions.

Results: The generated plasmas exhibited antimicrobial efficacy depending on the treatment time. The plasma jet was able to successfully kill *S. aureus* and *P. aeruginosa* in the treated area after 10 seconds. However, it did not exhibit an effect on *C. albicans* and *M. pachydermatis* until treatment was increased to 30 seconds. The DBD plasma source effectively eradicated the yeasts already after 10 seconds. In addition, it demonstrated significant antibacterial activity. Although the cross section of the DBD plasma source is bigger compared to the plasma jet, kill zones achieved were comparable. This indicates that the plasma is expelled from the jet by the gas flow and reaches an area that is larger than the nozzle contact.

Conclusions: The study showed that cold atmospheric pressure plasmas exhibit antimicrobial properties in vitro. Differences between the efficacies of the two plasma devices investigated could be found. Comprehensive studies will help to identify cell compatible plasma parameter sets with significant antimicrobial properties, resulting in the selective application of bioactive plasma for treatment of wound infections as well as other superficial skin infections such as dermatomycoses.

P168 | The role of skin mast cells in Sporothrix schenckii infection

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Mast cells (MCs) are abundant in the skin and other peripheral tissues, where they are one of the first immune cells to make contact with invading pathogens. As a result of pathogen recognition, MCs can be activated and release different preformed and de novo-synthesized mediators. Earlier studies suggested that MCs may play also a role in fungal infection. Here we investigated the response of MCs to Sporothrix schenckii, a dimorphic fungus that causes sporotrichosis, a subcutaneous mycosis found throughout the world in humans and other mammals. In murine bone marrow-derived MCs, *S. schenckii* yeasts induced the release of cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, IL-10, and IL-1β. However *S. schenckii* yeasts had no effect on MC degranulation as assessed by β-hexosaminidase activity assay and CD63 surface

expression by FACS analysis. In a model of cutaneous sporotrichosis, C57Bl/6 wt mice infected with *S. schenckii* yeasts developed markedly larger skin lesions than MC-deficient kit(W/W-v) or Cpa3- Cre; Mcl-1(fl/fl) MC-deficient mice. In addition, wt mice presented with a significantly increased inflammatory cell infiltrate after *S. schenckii* challenge compared to MC deficient mice. Cutaneous reconstitution of kit(W/W-v) mice with bone marrow derived MCs resulted in normalization of lesion size. These data suggest that mast cells contribute and regulate immune reactions of the host in response to *S. schenckii* infection.

P169 | Determination of different virulence factors of white and yellow strains of Trichophyton benhamiae from human and animal sources

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Introduction: Trichophyton benhamiae belongs perhaps to the most common zoophilic dermatophyte among children and adolescent in Germany causing acute and highly inflammatory cutaneous infections [1]. Previously it has been suggested that *T. benhamiae* occurs in 2 different colony types—yellow and white [2] but little is known on differences of virulence. In this study we investigated the pathogenicity of white (WS) and yellow strains (YS) of *T. benhamiae* from different origins using a skin equivalent and analyzed various enzyme activities.

Methods: 5 white (2 human/3 animal isolates) and 5 yellow strains (3/2) of *T. benhamiae*, identified by morphology and ITS sequencing, were used to infect 3D human skin equivalents (SE) followed by toxicity and inflammation analysis. Expression rates of pro-inflammatory cytokines and antimicrobial peptides (AMP) were determined by qPCR. SE were further subjected to histological analyses. For enzyme testing, microconidia suspension was added to yeast-extract medium alone or together with either sterilized hair or SE and incubated for 3 weeks at 30°C. Supernatant was used to determine activity of collagenase, elastase, and various hydrolases.

Results: Infection of the SE with human isolates of the *T. benhamiae* strains caused toxic and inflammatory reactions while effects of animal isolates were considerably more sever. Expression of cytokines and AMPs by SE was distinctly elevated after infection. YS exhibited higher secretion of enzymes for peptide degradation, phosphoric acid and fat-cleavage, as well as higher collagenase and elastase-activity while WS demonstrated enhanced secretion of sugar and glycoside degrading enzymes. In general, human isolates secreted higher amounts of all hydrolases.

Conclusion: This study underlines new aspects on virulence factors of both white (WS) and yellow strains (YS) of *T. benhamiae*. Co-culture analysis confirmed that animal isolates are more inflammatory; however, WS and YS do not differ significantly from each other. Enzyme secretion suggests that WS depend on sugars and YS on peptide, fat and phosphoric acid degradation. These findings give rise to the question whether the pathogenicity towards human cells contributes to different enzyme types compared to animal strains?

References: 1. Nenoff et al. J Dtsch Dermatol. 2014;12:571-81
2. Simons et al. J Med Microbiol. 2013;62:377-385.

P170 | Ex vivo inflammatory response of keratinocytes to infection with Trichophyton benhamiae

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Introduction: Keratinocytes represent the predominant cell type in the epidermis. Consequently, these cells are strongly involved in the first line of defence against invasion of pathogenic dermatophytes such as Trichophyton benhamiae. The induction of defensive inflammatory processes is mainly mediated by IL-8 and IL-6, both acting as pro-inflammatory stimuli to recruit immune cells. The present study demonstrates the pro-inflammatory cytokine response of human keratinocytes under infectious conditions. Since the prevalence of severe cutaneous infections caused by *T. benhamiae* increases but the pathogenicity mechanisms of this species are still not completely investigated, it is essential to understand the host reaction evoked by the dermatophyte.

Methods: Epidermal keratinocytes isolated from human foreskin were infected with *T. benhamiae* (DSM 6916) and cultivated for 24, 48 and 72 hours. Gene expression analysis of IL-6 and IL-8 was performed by quantitative real-time reverse transcription PCR (RT qPCR) and mRNA transcripts were visualized by RNA fluorescence in situ hybridization (FISH) using the QuantiGene® ViewRNA Cell Assay (Affymetrix Inc.). Protein levels of IL-6 and IL-8 secreted from keratinocytes were determined by ELISA and cell viability and toxicity were examined by cellular ATP content and LDH release.

Results: Human keratinocytes responded to co-cultivation with the dermatophyte at transcriptional levels after 24 hours. Infected cells showed significantly increased expression levels of IL-6 and IL-8 compared to non-infected cells. At protein level, the infection of the keratinocytes resulted in higher IL-6 secretion after 24 hours whereas IL-8 release was only moderately increased. A stronger induction of IL-8 secretion was observed after 48 hours. Infection with *T. benhamiae* caused severe epidermal cell damage revealed by elevated LDH release and reduced vital signs from infected keratinocytes.

Conclusions: Dermatophyte infections lead to inflammatory events by which epidermal cells like keratinocytes initiate the cutaneous defence against fungal invasion. In spite of the cytokine response, *T. benhamiae* was able to cause dramatic cell damage and loss of cells. These

findings underline the pathogenic relevance of the filamentous fungi that often appears to be underestimated.

P171 | In vivo risk assessment in a pilot study to analyze effects of wIRA treatment on skin tissue in a pig model

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Buruli ulcer, a debilitating skin and soft tissue infection caused by Mycobacterium ulcerans occurs most commonly in Africa, but also in Asia, the Western Pacific and America. The disease causing pathogen *M. ulcerans* produces a toxic mycolactone which inhibits normal immune cell reaction to wounding and induces necrotic cell death leading to the development of inflammatory ulcers. The only treatment options today are long term application of antibiotics, e.g. rifampicin and streptomycin or excision of the ulcer. Both treatment regimens are associated with severe side effects and high costs. Since *M. ulcerans* is temperature sensitive, hyperthermic treatment of the infection is a possible alternative. A lower risk of severe side effects and a cost reduction seems very likely. Lactobacterium *M. ulcerans* is located up to a depth of 2 cm in the tissue, wherefore hyperthermic treatment should heat up the subcutaneous tissue without burning the upper layers of the skin. Due to an emission wavelength of up to 1400 nm with cutting of the specific water absorption bands, water filtered near infrared (wIRA) devices induce a deeply penetrating thermal field in the skin without causing cross reaction with water molecules in the epidermal and dermal layers. The penetration depth and also the contactless applicability qualifies wIRA as possible therapeutic approach to treat patients with Buruli ulcer. To assess feasibility and possible risks of wIRA treatment for Buruli ulcer patients, we designed an in vivo pilot study on pigs with focus on measurement of surface temperature, examination of skin morphology and changes in protein profile. Measurement of surface temperature showed an increase of skin temperature up to 43°C during wIRA irradiation (106 mW/cm^2, 56 minutes, 780-1400 nm), reaching an irradiation induced temperature plateau after 15 minutes. Immunohistological examinations of punch biopsies revealed no risk for burning or other morphological changes of the applied irradiation regimen. Furthermore, we showed that wIRA irradiation with increase of skin temperature led to significant downregulation of MMP1 transcription. During protein profiling (ScioDiscover, Sciomics GmbH, Germany) 21 proteins were identified as targets for wIRA irradiation. These proteins are associated with survival, metabolism and stress response.

The herein shown preliminary results suggest that wIRA might be applicable for the treatment of Mycobacterium ulcerans infections.

P172 | *Staphylococcus epidermidis*-induced reduction of *S. aureus* skin colonization depends on the activation of Interleukin-1 receptor-induced innate immune signaling pathways

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

Objectives: We previously showed that secreted factors of the commensal *S. epidermidis* protect human and mouse skin against an infection with *S. aureus*. This work aims at elucidating the mechanism of this protective effect. We especially focus on the role of IL-1 receptor innate signaling since the IL-1R shares intracellular signaling molecules with pattern recognition receptors.

Materials & Methods: Using an in vitro skin infection model with primary human keratinocytes and human skin explants as well as an in vivo epicutaneous mouse skin infection model we analyzed the innate immune response induced by *S. epidermidis* and its effect on *S. aureus* skin colonization. Additionally, we used IL-1R deficient mice to examine the mechanism involved in the *S. epidermidis*-mediated modulation of *S. aureus* skin infection.

Results: We show that *S. epidermidis* is able to alarm keratinocytes by inducing the expression of interleukin-1 alpha, which itself is sufficient for the protective effect. Consequently, the *S. epidermidis*-mediated protection is lost in IL-1R-deficient mice.

Conclusion: In healthy skin *S. epidermidis*, as part of the skin microbiota, alarms keratinocytes and thus creates a protective environment which prevents *S. aureus* from colonizing the skin. Further studies will provide deeper insight into the mechanisms of the IL-1R-mediated modulation of the innate immune response, which reduces *S. aureus* skin infection.

P173 (OP02/05) | A humanized mouse model to study cutaneous leishmaniasis

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Leishmaniasis is a parasitic skin disease, with high rates of mortality and morbidity. In immune competent humans as well as C57BL/6 mice, infections with Leishmania (L.) major leads to Th1/Tc1 immunity with high levels of IL-12 and IFNgamma, whereas in

immunosuppressed humans and BALB/c mice, infections with L. major results in Th2/Treg/Th17-related immune responses with high levels of IL-4, IL-10 and IL-17. Up to date no vaccine against this human pathogen exists. Most of the knowledge described above has been gained by studying established mouse models. However, a transfer to the human system and to improve vaccine development to close the gap between mice and humans, so-called "humanized" mouse models may be helpful. To now analyze disease outcome in such "humanized mice", we transferred human stem cells into neonatal NOD-scid IL2rγnull (NSG) mice, which lack T, B and NK cells. Newborn mice were reconstituted with 1 × 106 $CD34^+$ cord blood-derived stem cells into livers. 16 weeks post transfer, reconstitution was assessed by flow cytometry. Effectively reconstituted mice were then infected in both ears with a physiologically relevant low dose of 1000 metacyclic L. major promastigotes mimicking the bite of a sand fly. Lesion volumes were measured weekly. Eight weeks post infection, mice were sacrificed. First, to confirm humanization we assessed population with human T and B cells as well as DC in blood, lymph nodes and spleen by flow cytometry. The numbers of human $CD3^+$, $CD8^+$ and $HLA^+/CD11c^+$ cells were comparable in all three tested organs, whereas the frequency of $CD4^+$ cells was strongly increased in spleens compared to blood and lymph node. Additionally, we enumerated infiltrating cells in spleens, livers, uninfected back skin, and infected ears by quantitative microscopic analysis. Tissue was stained and 5 representative high-power fields were enumerated for positively stained cells. Strong recruitment of human $CD45^+$, $HLA^+/CD11c^+$, $CD3^+$ T and $CD20^+$ B cells was observed, especially to spleens, liver and to the center of the granuloma compared to other tested organs (e.g. back skin, tissue surrounding granulomas). Further, we determined parasite burdens in infected ears and in spleens. Parallel to slowly increasing lesion volumes, humanized mice harbored rising numbers of local and systemic parasites. Body weights were measured weekly and remained stable throughout the infection. In addition, LN cells were restimulated with soluble Leishmania antigen (SLA) for 48 hours. The level of secreted human IL-10 was increased, whereas IFNgamma, IL-2, IL-6 and IL-8 were comparable to unstimulated controls. In summary, to study human cutaneous leishmaniasis and to aid vaccine development, utilization of humanized mice may be helpful and should be considered in the near future.

P174 | Optimization of a protocol for the assessment of living skin microbiota

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Human Skin, the largest organ of our body mediates information from the surrounding environment and provides the first line of defence against a wide range of pathogens. This barrier-function-role is both physical and immunological and it is supported by diverse microbial communities inhabiting the skin. The human microbiome plays a central role in host health and development. Indeed, dysbiotic shifts in its composition have been linked to a wide variety of diseases, such as atopic dermatitis, psoriasis, asthma, colitis, obesity, etc. Culture-independent methods to characterize the microbiota have gained increasing interest, due in part to affordable and accessible sequencing and analysis platforms. Compared to culture-based techniques, DNA sequencing of the bacterial 16S ribosomal RNA gene or whole metagenome shotgun sequencing provide more accurate microbial community characterizations. However despite the great improvement in microbiota investigation using NGS technology, it still has the drawback of considering both living and dead bacteria. The main aim of this study is to optimize a microbiome isolation and characterization protocol in order to better discriminate living and dead bacteria. A mock community has been analyzed using different DNA isolation kits with an adaptation of the protocol in order to get rid of dead bacteria and bacteria free DNA. The optimization includes furthermore the depletion of host contaminating DNA which represents an issue especially when dealing with samples enriched in host cells material such as skin and lung samples. We validate in this study a method for bacterial DNA processing allowing a better representation of the living microbiota with a clear decrease of the host contaminating DNA. Further investigations are needed for better characterization of living microbiomes at different body sites.

PHARMACOLOGY

P175 | Olive oil-derived phenols exert effective anti-inflammatory properties by interference with the NF-kB pathway and TSLP production in human keratinocytes.

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Introduction: Extra virgin olive oil (EVOO) is rich in diverse phenolic compounds, including hydroxytyrosol (HTy) and hydroxytyrosyl acetate (HTy-Ac) that have shown a wide range of beneficial properties. However, the potential benefits of these EVOO bioactive compounds on the inflammatory balance of the skin have not been elucidated yet. AIM: The present study aimed to evaluate the anti-inflammatory properties of HTy and HTy-Ac in an in vitro model of human keratinocytes and to analyse the mediators and molecular mechanisms involved.

Methods: Human keratinocytes were isolated from the skin of healthy donors. Cells were preincubated with HTy-Ac or HTy, then stimulated with IL-1B or TLR3-l for different times. TSLP in the

supernatant was quantitated by ELISA, and TSLP, as well as other genes related to the inflammatory response, were likewise determined by RT-qPCR. IkB degradation and NF-kB translocation to the nucleus were studied by western blotting, and the ChIP assay served to examine recruitment of NF-kB to the TSLP and IL-8 promoters, respectively.

Results: EVOO phenols significantly reduced TSLP and IL-8 in human keratinocytes. HTy and HTy-Ac counteracted induced NFkB activity through prevention of IkBa degradation and p65 nuclear translocation and neutralized the effect of IL-1B induced recruitment of NF-kB on promoters of TSLP and IL-18 genes.

Conclusion: Our data demonstrate that EVOO phenols modulate the inflammatory process in human keratinocytes by decreasing TSLP production and gene expression and other proinflammatory molecules, through interference with the NF-kB pathway. Therefore, these EVOO polyphenols may be used as promising compounds for the management of inflammatory skin diseases.

P176 | Arsenic trioxide decreases lymphangiogenesis by inducing extrinsic and intrinsic apoptosis and inhibition of important lymphatic endothelial cell receptors

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Purpose: Lymphangiogenesis is a crucial step in the progression of cancer. Formation of new lymphatic vessels provides an additional route for tumor cells to metastasize. Therefore, influencing lymphangiogenesis is an interesting target in cancer therapy. Signaling via the vascular endothelial growth factor receptor-2/3 (VEGFR-2/3), Lyve-1 and Tie-2 pathways are critical for lymphangiogenic responses. Arsenic trioxide (As2O3), which is used as an effective treatment against relapsed acute promyelocytic leukemia, is characterized by low cytotoxicity. As As2O3 promotes anti-angiogenic effects on endothelial cells, we hypothesized that As2O3 may have impact on lymphangiogenesis through the inhibition of the important endothelial cell receptors VEGFR-2/3, Lyve-1 and Tie-2.

Methods: Human lymphatic endothelial cells were cultured in vitro and treated with or without As2O3. Effects of As2O3 on proliferation, apoptosis and expression of important endothelial receptors were analyzed mainly by BrdU-Assay, cell death assay, caspase activity assays, cytochrome c assay and immunoblotting. In vitro angiogenesis was investigated using the matrigel tube formation assay.

Results: As2O3 inhibited cell proliferation in a concentration-dependent manner. In our study we found that As2O3 induced apoptosis by activating caspase- 8 and 9, cytochrome c release and down-regulating the anti-apoptotic proteins cIAP-2 and survivin. Furthermore, we could demonstrate an inhibition of the formation of lymphatic capillary like structures by As2O3 treatment. In addition, we demonstrated that As2O3 significantly inhibited protein levels of VEGFR-3, Tie-2 and Lyve-1 whereas VEGFR-2 expression was unaffected after treatment with As2O3. Additionally, mRNA levels of VEGFR-3 and Lyve-1 were suppressed after treatment with As2O3.

Conclusions: In conclusion, our results provide for the first time clear evidence, that As2O3 has distinct anti-lymphangiogenic effects mainly by inhibition of the endothelial VEGFR-3, Tie-2 and Lyve-1 and activating both the extrinsic and intrinsic apoptotic pathways.

P177 | The effect of monomethyl fumarate on human blood neutrophils

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Fumaderm®, a mixture of fumaric acid esters, has been used to treat psoriasis for more than 20 years. In 2008, it was shown that monomethyl fumarate (MMF), the active metabolite of Fumaderm®'s main component dimethyl fumarate binds to hydroxycarboxylic acid receptor 2 (HCA2). As neutrophils play a major role in the pathogenesis of psoriasis, we investigated the effects of MMF on human neutrophils.

By flow cytometry we studied neutrophil counts in the blood of 13 psoriasis patients taking Fumaderm®. 92% (12 out of 13) of patients showed a mean decrease of 75% (SD 19%) in peripheral neutrophils in the first three months after starting treatment. Next we assessed HCA2 expression by human blood leukocytes and found that in contrast to T cells, B cells and eosinophils, neutrophils expressed HCA2 protein on their surface. In vitro stimulation of freshly isolated neutrophils revealed that HCA2 mRNA expression could be upregulated by interferon-γ. The HCA2 receptor agonists MMF and nicotinic acid did not change HCA2 transcript levels. In contrast, nicotinic acid and MMF were able to reduce HCA2 surface protein levels when analysed by flow cytometry, suggesting that MMF may cause agonist-induced internalisation of HCA2. To determine if the neutrophil drop seen during fumarate treatment was due to the induction of apoptosis, we stimulated human neutrophils with pharmacological relevant concentrations of MMF in vitro and assessed the rate of apoptosis using Annexin V staining. We found that MMF did not increase the rate of apoptosis in neutrophils when compared to vehicle-stimulated controls. Using a Proteome Profiler Assay we assessed if MMF could induce any apoptosis-related signalling pathways in neutrophils. In agreement with our Annexin V results we found that MMF did not induce phosphorylation of any apoptosis related-proteins. HCA2 agonists have been reported to inhibit the forskolin-induced accumulation of intracellular cyclic adenosine monophosphate (cAMP). Thus we investigated if MMF could have a similar effect, however we found that MMF caused a small non-significant decrease in cAMP in forskolin activated neutrophils. We

also determined that MMF was unable to induce a calcium flux in neutrophils.

These data indicate that the drop in peripheral blood neutrophils observed in Fumaderm®- treated psoriasis patients is not due to direct induction of neutrophil apoptosis. Therefore, other indirect mechanisms that alter the size of the blood neutrophil population must exist. The internalisation of HCA2 in response to MMF by neutrophils suggests that there are downstream signalling events occurring but that these do not appear to be linked with changes in the second messengers cAMP and calcium.

PHOTOBIOLOGY

P178 | Splice variants of the endonucleases XPF and XPG contain residual DNA repair capabilities and could be a valuable tool for personalized medicine

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The two endonucleases XPF and XPG are essentially involved in nucleotide excision repair (NER) and interstrand crosslink (ICL) repair. Defects in these two proteins result in severe diseases like xeroderma pigmentosum (XP). We applied our newly CRISPR/Cas9 generated human XPF knockout cell line with complete loss of XPF and primary fibroblasts from an XP-G patient (XP20BE) to analyze until now uncharacterized spontaneous mRNA splice variants of these two endonucleases. Functional analyses of these variants were performed using luciferase-based reporter gene assays. Two XPF and XPG splice variants with residual repair capabilities in NER, as well as ICL repair could be identified. Almost all variants are severely C-terminally truncated and lack important protein-protein interaction domains. Interestingly, XPF-202, differing to XPF-003 in the first 12 amino acids only, had no repair capability at all, suggesting an important role of this region during DNA repair, potentially concerning protein-protein interaction. We also identified splice variants of XPF and XPG exerting inhibitory effects on NER. Moreover, we showed that the XPF and XPG splice variants presented with different inter-individual expression patterns in healthy donors, as well as in various tissues. With regard to their residual repair capability and dominant-negative effects, functionally relevant spontaneous XPF and XPG splice variants present promising prognostic marker candidates for individual cancer risk, disease outcome, or therapeutic success. This merits further investigations, large association studies, and translational research within clinical trials in the future.

PRURITUS

P179 | Bad vibrations: insights into the mysterious on/off itch and erythema during whole body vibration exercise

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Background: Whole body vibration (WBV) is a common training method using high strain rates and fluid flow to improve muscle and bone strength. Although "post-exercise reactive hyperaemia" has been reported in some studies, the appearance of itch and/or a rash during WBV exercise has not been described yet.

Objectives: (i) To investigate possible pathophysiological mechanisms underlying the development of itch and erythema induced by WBV, (ii) determine the frequency of this phenomenon and (iii) define the potential impact of individual factors and device settings on its appearance.

Methods: Questionnaires for WBV users and staff members, respectively, were distributed in 4 training centres in Switzerland and Germany. 3 healthy WBV users who had experienced intense itch and erythema during WBV were reexposed in a standard exercise (ten 1-minute repetitions; 20 Hz; 3 mm amplitude; 1-minute breaks) and rated itch intensity on a 0-10 numerical rating scale (NRS) at 1-minute intervals. Potential skin changes were examined by two experienced dermatologists.

Results: 67 WBV users and 6 staff members completed the questionnaires. 17 (25%) users reported itch with a mean intensity of 5 (NRS) and/or erythema. Symptoms appeared mostly during the first and persisted throughout all sessions. Staff members usually observed an erythema within 5 minutes after starting and a complete remission 5 minutes after termination of WBV. All symptomatic users had trained with a side-to- side alternating device. In healthy users the mean interval before itch onset was 2 minutes, usually preceding erythema development by 2 minutes. Itch reached its maximum (mean NRS 8) after 8 minutes, decreased within 3 seconds after termination and reappeared within 3 seconds when restarting WBV. In none of the individuals urticarial lesions or systemic symptoms were observed.

Discussion: The instant on/off nature of itch and the lack of urticarial lesions indicate that this phenomenon is rather mediated by vibration-responsive low-threshold mechanoreceptors than by degranulation of skin mast cells as seen in vibratory urticaria. As erythema appeared already during WBV, the term "post-exercise reactive hyperaemia" does not seem to be suitable. Moreover, a recent study indicated that low frequency vibration does not lead to vasodilation but to ejection of venous blood from muscle vessels potentially explaining why itch and erythema in WBV are not clearly linked compared to itch (or pain) observed in reactive hyperaemia due to vasodilation.

P180 (OP05/06) | The molecular signature of chronic prurigo

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Chronic prurigo (CPG) is a highly pruritic disease, characterized by hyperkeratosis, secondary scratch lesions, papules, plaques and/or nodules. However, only limited data exist on the underlying molecular signature of CPG and hitherto no transcriptomics data have been reported. Therefore, we aimed to provide a comprehensive map of the global mRNA expression profile of CPG. Matched lesional (LE) and non-lesional (NL) biopsies were collected from nine CPG patients and preserved by FFPE. Total RNA was extracted, and global mRNA expression profiles were generated by Affymetrix HuGene 1.0 ST microarrays. Overall, 728 mRNAs were differentially expressed (>2-fold difference, $P<.01$) between LE and NL samples (451 up- and 277 down-regulated), including DEFB4A, SERPINB3/4, SPRR2A/B, S100A7/9, LCE3D/E, PI3, KRT6A/B, IVL, and FLG (all more than 10-fold up-regulated in LE), and TSPAN8, RBP4, DCD, LEP, ADIPOQ, LPL, TIMP3, NPY1R, TNMD, KRT19, FABP4, and DPT (down-regulated in LE). Based on functional annotation clustering, the up-regulated genes were highly enriched for keratinocyte differentiation, keratinization, and inflammation associated genes. Ingenuity Pathway Analysis identified the oncostatin M (OSM) pathway as potentially involved in the pathogenesis of CPG. Additionally, most of the CPG patients could be classified into two subsets: one (3 patients) with a strong epidermal signature (i.e. high in FLG, LOR and other keratinocyte-specific genes), and the other (5 patients) with a weaker epidermal profile, perhaps suggesting different degrees of disease severity or different underlying pathomechanisms in CPG. Interestingly, although CPG is often present in patients with an atopic background, a comparison of our data to published mRNA expression studies on atopic dermatitis (AD) and psoriasis (PSO) showed that the CPG signature resembles PSO more than AD. Taken together, our analyses of the molecular signature of CPG have uncovered possible subsets of the disease enabling differential diagnosis, and uncovered the oncostatin M pathway as potentially associated with the pathogenesis of CPG.

P181 | GEHIS (German Epidemiological Hemodialysis Itch Study): Results of a follow up study on chronic itch in hemodialysis patients

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The German Epidemiological Hemodialysis Itch Study (GEHIS) is a representative cross-sectional cohort study including 860 hemodialysis (HD) patients in 25 dialysis units in Germany. It showed 25.1% (n=217) of the patients to suffer from current chronic itch (CI) (point prevalence) and 27.2% (n=234) to have suffered from CI in the previous year (1-year prevalence). A follow-up study in 2017 aimed to investigate the course of CI 3 years later in those HD patients who had suffered from CI before. Sociodemographic data, current and previous CI, quality and intensity of CI (visual analog scale, VAS), health-related quality of life (HRQOL) using the SF-12 and itch-related quality of life using ItchyQOL were assessed. Altogether, 234 HD patients had been suffering from CI at baseline. 130 HD patients could not be investigated, most of them (85 HD patients, 36.3%) because they had died. 23 HD patients (7%) were meanwhile engrafted, the other ones could not participate for other reasons such as e.g. change of the HD unit. 104 CI patients were investigated which corresponds to a response rate of 82.54%. 54.8% (n=57) were male. The mean age was 64.78 years (SD: 13.29 years). In 50% (n=52) CI had stopped, 50% (n=52) still suffered from CI. The intensity of CI had not changed significantly (mean VAS 4.21 in 2013 vs. 4.13 in 2017). As at baseline, the three most frequent localizations of CI were the back, the legs and the scalp. Significant changes were detected in HRQOL. The decrease in the score of the physical subscale of the SF-12 was significantly lower in HD patients that did not suffer from CI any more (n=52) compared to those with persisting CI or compared to patients with no history of CI, indicating a better HRQOL (−0.26 vs. −3.64 and −4.45). ItchyQoL did not show any significant changes in the patients with persisting CI (total score: 2.1 in 2013 vs. 2.16 in 2017). This is the first study to follow up CI in HD patients of a representative cohort. Our data confirm CI to be an enduring symptom in HD patients significantly influencing their well-being and resembling a chronic burden.

P182 | Gene expression profiling in chronic prurigo of nodular type

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Chronic prurigo of nodular type (PN) develops from chronic pruritus due to continuous scratching that leads to multiple itchy nodules. Although chronic prurigo shows a high negative impact on daily life quality, effective therapies are still not available. A deeper insight into affected pathways and involved genes may help to define new therapeutic targets.

Therefore, we performed global gene expression profiles in five PN patients and matched healthy controls (HC). Biopsies were obtained from lesional/pruritic (LP) and non-lesional/non-pruritic (NL-NP) skin of patients and from healthy skin of controls (HC). Expression profiles were generated using Affymetrix Human Gene

1.0 ST Arrays. Due to the small sample size data processing was done using the R/Bioconductor package limma. Deregulated genes were defined with an absolute log2-fold change >1 and a *P*-value <.05. For pathway analysis we made use of Reactome, a free open source database.

A first analysis revealed most deregulated genes (DEG) between L-P and unaffected skin of patients (NL-NP; n=276) and controls (HC; n=376). NL-NP and HC showed similar gene expression profiles with only a small number of DEGs (n=58). Different settings for overlapping DEGs like L-P/NL-NP vs HC (n=9) and L-P vs. NL-NP/HC (n=223) were calculated. Pathway analysis revealed DEGs of the latter comparison to be enriched in various pathways like keratinization, formation of the cornified envelope, RUNX1 regulation of keratinozyte differentiation, signalling by interleukins, and others. Further analyses of the data are underway and may provide an even deeper knowledge of molecules and pathways involved in PN pathology.

P183 (OP06/03) | Blocking the neurokinin 1 receptor in chronic prurigo reveals peripheral mechanisms

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Blocking the neurokinin 1 receptor (NK1R) in chronic prurigo has recently been shown to reduce pruritus intensity. Both, central effects involving spinal dorsal horn neurons and peripheral effects involving cutaneous components are discussed as underlying mechanisms. In human skin NK1R may initiate several inflammatory reactions like mast cell activation and expression of pro-inflammatory cytokines by keratinocytes.

Within this study we therefore investigated in vivo and in vitro peripheral effects mediated by the NK1R antagonists aprepitant and casopitant. For the in vivo study 13 patients suffering from chronic prurigo of nodular type (PN) received an oral four weeks treatment with aprepitant (80 mg/d). Clinical and immunohistochemical parameter were assessed before and after treatment. Furthermore, biopsies were obtained from lesional skin (pruritic nodules) before and after treatment for analysis of NK1R expression and activation of potential downstream targets. Studies were continued in vitro using human keratinocytes (NHEKs). Cells were stimulated using the NK1R activating substance P (SP) with or without pretreatment with aprepitant or casopitant. Again, the effect of NK1R antagonism on downstream molecules was assessed.

Treatment with aprepitant reduced significantly pruritus intensity in PN patients. This was not reflected by histological changes what may be due to the short treatment period in which improvement but not complete healing of lesional nodules was achieved. Immunohistochemistry revealed altered expression of some inflammatory marker suggesting a peripheral therapeutic effect. Epidermal NK1R expression was higher in PN patients compared to matched healthy controls (n=10); after treatment with aprepitant it increased even more. We speculate that the upregulation may be needed to overcome at least in parts the long lasting blockage by aprepitant. First analyses of NK1R antagonism in keratinocytes in vitro revealed reduced expression of pro-inflammatory cytokines. Additionally we analysed expression and activation of ERK1/2 that can be activated by NK1R and may initiate cytokine expression. SP induced activation/ phosphorylation of Erk1/2 was significantly reduced by both, aprepitant and casopitant. This was confirmed in vivo as PN patients showed reduced Erk1/2 expression and phosphorylation after treatment with aprepitant.

In sum, altered receptor expression, reduced MAPK activation in vivo and in vitro and decreased cytokine expression suggests a peripheral mechanism on keratinocytes for the observed antipruritic effect of NK1R antagonism.

TUMOR BIOLOGY

P184 | The expression of chondroitin sulfate proteoglycan 4 (CSPG4) is decreased in BRAF-mutant induced drug-tolerant melanoma cells

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Background: Acquired resistance to PLX4032, a selective inhibitor of mutant BRAF, remains the major obstacle in treating patients with metastatic melanoma. Induced drug-tolerant cells (IDTCs), characterized by an increase in CD271 expression, constitute a state preceding permanent drug-resistance. Chondroitin sulfate proteoglycan 4 (CSPG4) is a multifunctional transmembrane proteoglycan, involved in spreading, migration and invasion of melanoma. We hypothesize that targeting CSPG4 on IDTCs may prevent the development of acquired drug-resistance. Here, we investigate the expression of CSPG4 in BRAF-mutant melanoma cells exposed to PLX4032.

Methods: BRAF-mutant WM164 melanoma cells were exposed to PLX4032 in order to generate IDTCs. CD271 expression was monitored as a marker of IDTCs by flow cytometry using an anti-CD271-APC mAb. CSPG4 expression on melanoma cells before, during and after exposure to PLX4032 was evaluated by FACS and immunofluorescence (IF) microscopy using an anti-CSPG4 mAb.

Results: Exposing WM164 cells to PLX4032 for 9 days led to IDTCs, which were characterized by elevated CD271 expression compared to non-treated cells and by morphological changes

such as an elongated shape or dendrite-like structures. A lower mean fluorescence intensity of the CSPG4 signal was found in IDTCs (1594.628.7) compared to untreated cells (2829.573.9). Interestingly, the percentage of CSPG4-positive cells determined by FACS did not vary significantly between exposed and unexposed cells. IF microscopy confirmed a decreased amount of CSPG4 on the cell surfaces of IDTCs.

Conclusion: Exposure to PLX4032 at sub-lethal concentrations did not influence the number of CSPG4-positive cells but led to a decrease of CSPG4 expression on IDTCs. These results might indicate that the inhibition of mutant BRAF influences the expression of CSPG4. This provides the basis for further investigation of the role of CSPG4 in the development of permanent drug-resistance in melanoma cells.

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P185 (OP05/03) | Effect of selective Gq/11-inhibition on malignant melanoma

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G-protein-coupled receptors (GPCRs) comprise a large family of cell-surface receptors that transduce signals through interaction with heterotrimeric G-protein subunits (Gα,Gβ and Gγ). The aberrant expression, overexpression or signal reprogramming of GPCRs and G-proteins have been linked to cancer initiation, tumor cell growth, metastasis and angiogenesis. Frequent somatic mutations of Gnaq and Gna11 were found in uveal melanomas of humans thereby identifying these G proteins as potential oncogenes in human neoplasia. As a future perspective the inhibition of wild-type and/or mutated Gq-GPCRs as well as constitutively active Gαq mutants may represent an effective molecular intervention to target oncogenic signaling. Currently there are only two known selective Gq/11-inhibitors, FR-900359 and YM-254890, which are of potential interest to target oncogenic signaling. Here we analyzed the role of GPCR-Gαq signaling in primary and transplantable Hgf- Cdk4R24C mouse melanomas using FR-900359 in vitro and in vivo. We also examined the effect of FR-900359 and YM-254890 on human uveal melanoma cells carrying wild-type or mutated Gnaq genes. We found that oncogenic mutations in Gnaq/11 appear to be selected in primary Hgf-Cdk4 mouse melanomas. All transplantable Hgf-Cdk4 mouse melanomas including HCmel12 and HCmel3 carried oncogenic mutations in Gnaq/11 genes. FR-900359 inhibited the proliferation of the GnaqQ209L-mutated HCmel12 mouse melanoma cell line and abrogated ERK activation. FR-900359 and YM-254890 both reduced the proliferation and ERK activation of Gnaq-mutated uveal melanoma cells but had no effect on Gnaq wildtype uveal melanoma cells. Future studies will have to address how exactly Gαqcoupled receptors transduce proliferative signals in melanoma cells. They are known to stimulate multiple second-messenger systems and are also able to transactivate tyrosine kinase growth factor receptors. In ocular melanomas, Gαq controls cell proliferation by transcriptional regulators such as JUN, FOS and YAP. How these pathways contribute to growth and migration of malignant melanoma cells still remains to be elucidated.

P186 (OP06/06) | A Drosophila melanogaster model for melanoma drug screening

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Here we developed a Drosophila melanogaster model of the melanoma BRAFV600E mutation for drug screening. In this model, the expression of the constitutive active isoform of human BRAF can be targeted to different tissues of interest, using the GAL4/UAS expression system. We examined whether ubiquitous or tissue specific expression of BRAFV600E led to specific phenotypes. The expression of BRAFV600E by the strong retinal-specific GMR promoter as GMR-GAL4 driver led to semi-lethality in the pupal developmental stage and adult "escapers" showed a rough eye phenotype. By use of mutant BRAF kinase inhibitor vemurafenib and MEK1/2 inhibitor cobimetinib the pupal viability was rescued up to 22.3% (combined treatment) compared to 4.3% in the DMSO-treated group, with single-agent treatment being slightly less effective. To further improve this system, because the rough eye phenotype could not be rescued, the esg-GAL4 driver was used which is expressed in the gut and therefore provided optimal activity of ingested inhibitors. Treatment by vemurafenib and cobimetinib highly significantly rescued the transition from larval into the pupal stage in up to 42.3% (combined treatment) compared to 0.7% in the DMSO-treated group. By use of a mutant Drosophila RAF (dRaf) model there was no effect for the mentioned BRAF inhibitor but for the MEK1/2 inhibitor. Among additionally tested inhibitors in the dRaf system, a recently described VEGF receptor inhibitor showed promising results. Taken together, our mutant BRAF/dRaf models proved to be useful for validating the efficacy of vemurafenib and cobimetinib treatment and may now be used for drug screenings of other candidate substances. Moreover, Drosophila may also be used to validate the biological relevance of other recently identified genetic variants of melanoma.

P187 (OP03/02) | Type I IFN responsiveness of melanoma is cell state dependent and can be both harnessed and suppressed to enhance oncolytic virotherapy efficacy

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Oncolytic virotherapy is a new promising approach to treat malignant melanoma. Tumor cells are often permissive for viral infection and oncolysis due to their active metabolism and decreased responsiveness to type I interferons (IFN-I). However, also IFN-I responsive tumors may be suitable targets for oncolytic virotherapy, as local IFN-I responses have often been associated with anti-tumor immunity. The underlying mechanisms accounting for the differences in IFN-I responsiveness of melanoma are poorly understood. The aim of this work was to analyze the responsiveness of a collection of human and mouse melanoma cell lines to IFNI utilizing an oncolytic Semliki Forest virus expressing EGFP (SFV-VA7-EGFP). 16 human melanoma cell lines with a spectrum of phenotypes ranging from very melanocytic ($MITF^{high}$) to poorly differentiated ($MITF^{low}$) were screened for their IFN-I responsiveness by treatment with varying concentrations of IFN-I followed by infection. The infection kinetics were monitored with fluorescence and bright field microscopy over 72 hours, after which the net result of cell proliferation was quantified using crystal violet staining. Following IFN-I pretreatment, healthy primary melanocytes were readily protected from infection, whereas all melanoma cell lines had, to varying degree, lowered antiviral type I IFN responsiveness. Melanoma cell lines, which had retained partial responsiveness to IFN-I displayed a basal IFN-I signature in a bioinformatic analysis. Interestingly, the one quarter of the melanoma cell lines with poorest IFN-I responsiveness were all melanocytic ($MITF^{high}$), suggesting a potential link between the differentiation status and the responsiveness to type I IFNs. Supporting the hypothesis, MITF overexpression utilizing a tet-ON system in $MITF^{low}$ Mamel65 human melanoma cell line completely abrogated their type I IFN responsiveness allowing productive SFV-VA7-EGFP infection and oncolysis. To test the hypothesis that both suppressing and harnessing the type I IFN responses may be utilized to benefit oncolytic virotherapy, we treated HCmel12 mouse melanomas with SFV-VA7-EGFP in combination with antibodies targeting either the type I IFN receptor or an immunosuppressive PD1 receptor on T cells. While both approaches were found to enhance oncolytic virotherapy efficacy and type I IFN-receptor deficient melanomas were eradicated in Rag-knockout mice, local IFN-I signaling blockade in the tumors before virus administration resulted in marked toxicities suggesting peripheral spread of the infection.

P188 | MAPK inhibitor resistance is associated with intrinsic TAp73 down-regulation and enhanced platinum-based drug sensitivity in malignant melanoma

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Metastatic melanoma patients benefit from the recently approved targeted BRAF inhibitor (BRAFi) and dual MAPK inhibitor (MAPKi) therapy; however the development of drug resistance limits the long-term efficacy. Furthermore, treatments with cytostatic drugs were shown to be not effective as first line melanoma therapy. Different isoforms of the p53 homologue p73 are known to have cell-type specific functions in tumor cells. In melanoma cells, the functional role of the TAp73 isoform is widely unknown.

We found that melanoma cells with acquired resistance to the MAPKi treatment have a reduced basal TAp73 level and are more susceptible towards carboplatin and cisplatin treatment than treatment-naive melanoma cells. In line with this, downregulation of endogenous TAp73 expression enhanced the sensitivity towards the platinum-based drugs whereas TAp73 overexpression reduced the sensitivity to these drugs. In addition, we could determine the TAp73α isoform as an abundantly expressed intrinsic p73 transcript variant in treatment-naïve melanoma cells. Moreover, Its expression negatively correlated with the susceptibility of these cells to platinum-based drugs. Furthermore, we could show that TAp73 level influence the cisplatin induced accumulation of DNA damage and the expression of specific DNA damage repair associated genes.

Our data demonstrate a pro-survival impact of the intrinsic TAp73 expression for melanoma cells under platinum-based drug treatment. Furthermore, we propose that TAp73 expression can be used as a marker to identify the subset of MAPKi resistant melanoma with enhanced susceptibility to cisplatin or carboplatin treatments.

P189 | JunB—a potential downstream mediator of TNF signaling in cytokine-induced cancer cell senescence?

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Today cancer treatment has extended its focus from surgery or chemotherapy to tumor immunotherapy, which has gained increasing relevance. An efficient cancer immunotherapy shows cancer cell apoptosis or killing. In addition, it may induce a stable growth arrest. This stable growth arrest may result from senescence induction,

a process normally associated with aging or cell stress caused by oncogenes.

We recently found that senescence can be induced by the Th1 cytokines interferon gamma (IFNγ) and tumor necrosis factor (TNF) in vitro and in vivo. This cytokine induces senescence (CIS) was strictly depending on the induction of the tumor suppressor p16INK4a, which in turn inhibits the phosphorylation of retinoblastoma protein (Rb1), a signaling molecule that prevents the cancer cells from entering the cell cycle. Since neither IFNγ nor TNF directly targets p16INK4a, we searched for other downstream signals for the activation of p16INK4a.

The activator protein 1 (AP-1) family member JunB is a potential candidate to mediate this signal transduction, since the transcription factor is a known target of the TNF signaling and was shown to activate the transcription of p16INK4a. It is unclear whether JunB can bind to the p16INK4a promoter as a homodimer or if binding requires the association with other AP-1 members. In order to investigate the CIS mediated signaling pathway in detail, we studied its effects on primary murine cell lines isolated from the pancreas of RIP Tag2 mice, where the SV40 large T antigen 2 (Tag2) is expressed under the rat insulin promoter (RIP) causing an inhibition of p53 and Rb1. In addition, we investigated murine CT26, Lewis lung carcinoma and the human sarcoma cell line A204. In all four cancer cell lines CIS induced a 2-3 fold upregulation of JunB protein up to 8 hours after Th1 cytokine treatment.

To analyze the functional role of JunB we knocked down JunB in RIP-Tag2 cells with shRNA. Furthermore, we investigated the JunB binding partner during CIS by introducing a Strep-FLAG-TAP-tagged JunB construct in A204 cells.

First data show that the cytokine concentrations of TNF and IFNγ, which induce CIS in untransfected cells, cause cell death in the transfected cells, suggesting that cells overexpressing the Strep-FLAG-TAP-tagged JunB construct are highly sensitive to TNF. We therefore stimulated the transfected cells with IFNγ alone, asking whether the transfection compensates for the TNF signaling. Indeed, low doses of IFNγ resulted in a stable growth arrest, suggesting that JunB mediates the TNF signals required for CIS.

Thus, overexpression of JunB tagged to the Strep-FLAG-TAP construct sensitizes A204 cells towards TNF signal needed for the senescence induction. Since TNF cannot be administered systemically in the clinic, targeting JunB might be an alternative way to activate the TNF signaling pathway required for the induction of CIS in cancer cells.

P190 | Cytokine-induced senescence (CIS) drives cancer cells to polarize naive macrophages

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Cellular senescence plays an important role in tissue development, homeostasis and cancer control. It is an intrinsic growth control mechanism that prevents transformed pre-malignant lesions to progress into overt malignancy. In addition to endogenous stress, cytokine signals delivered by the immune system can induce senescence in human and mouse cancer cells. This cytokine induced senescence (CIS) is mediated by the Th1 cytokines IFNy and TNF, and induces senescence in murine ß-cancer cells and many other murine and human cancers in vitro and in vivo. It is characterized by a G0/G1 arrest of the cell cycle, a stable proliferation arrest and induction of senescence associated ß-galactosidase activity, thereby promoting aging or tissue degeneration and possibly restraining cancer.

Yet by the secretion of the senescence associated cytokine and chemokines, senescent cancer cells may remain a potential harm. Here, we analyzed the molecular changes that CIS induces in cancer cells, to determine whether and, if so how, senescent cancer cells can be cleared by the immune system. In the present study, we found that CIS downregulates the expression of CD47, a surface marker that prevents recognition of cells by the immune system, on senescent beta cancer cells in vitro. In addition, senescent beta cancer cells showed an increased surface expression of phosphatidylserine, a recognition marker for immune cells. We then analyzed the senescence associated secretory phenotype (SASP) and its influence on cycling tumor cells as well as bone-marrow derived macrophages. SASP components, like CCL2 and CCL5, increased non-senescent tumor cell proliferation. The pro-inflammatory SASP is highly attractive to immune cells and recruits bone marrow derived macrophages. However, these macrophages polarize towards a pro-tumorigenic M2 phenotype. Such M2-like macrophages are characterized by lack of iNOS and concurrent increase in arginase1 protein levels. These data underline that the removal of senescent cancer cells from the organism may be an important cancer-protective mechanism. Taken together, modulating the secretome of senescent cancer cells may change the tumor microenvironment, trigger different anti-tumor immune responses and directly affect the immune responses to nonsenescent cancer cells.

P191 | Stromal hyaluronan supports melanoma cell proliferation and angiogenic potential

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The tumor-microenvironment (TME) is a complex interaction network of tumor and local cells, infiltrating immune cells, extracellular matrix (ECM) components, and soluble mediators. Current research has focused on the TME with respect to promotion of tumor angiogenesis, migration, and proliferation. Hyaluronan (HA) is an abundant glycosaminoglycan polymer of the ECM contributing to its structure and functionality. Studies revealed a correlation of

increased deposition of HA in TME with poor prognosis for breast cancer patients (Auvinen et al., 2000). Stromal HA is produced by three hyaluronan-synthases (HAS): HAS1, HAS3, but mainly HAS2. We proved that the high amount of HA is not produced by the tumor cells themselves but they stimulate surrounding fibroblasts—also called cancer-associated fibroblasts (CAF)—to elevate their HA production (Willenberg et al., 2012). Silencing of HAS2 in vitro showed no compensatory upregulation of HAS1 or HAS3. The membrane bound receptors RHAMM and CD44 interact directly with stromal HA, enabling HA cell attachment, cell motility and also initiate signal transduction upon binding.

Using 2D culture of human malignant melanoma (MM) cells on decellularized matrices deposited by dermal fibroblasts (Amatangelo et al., 2005) we found that depletion of HA results in decreased HA-synthesis and HA-degradation by the tumor cells. Gene expression array of those cells revealed that removal of HA from the substrate results in an expression pattern with decreased proliferative and angiogenic potential and strong downregulation of genes associated with invasion and metastasis, that was confirmed also in MM migration experiments on such decelluarized matrices. When growing MM cells in cross-linked 3D collagen networks functionalized with different HA molecules (Sapudom et al., 2017) we found a $CD44^{-}$ dependent pro-proliferative effect of HA of low molecular weight (LMWHA, 34 kDa) whereas HA of high molecular weight (HMWHA, 1200 kDa) had no effect on tumor cell proliferation.

To investigate the role of HA for solid tumor growth in vivo and to circumvent embryonic lethality, we developed an inducible, conditional HAS2- knockout mouse with inducible, decreased HA production. We established the CRE-ERT2 induction procedure and the validation of HAS2 knockout in vivo by HA-quantitation and size analysis, RT-qPCR and immunofluorescence microscopy. Up to 80% reduction of HA deposition in skin, with preferential loss of high molecular weight HA has been confirmed for at least 21 days post-induction. B16F10 melanoma cells were injected into the skin and tumor growth and gene expression in the developing tumors were analyzed. Tumors in mice with induced HAS2-knockout showed minor differences in primary size and a significant reduction of CD31 expression. These findings support in vitro data, where proliferation was dependent on LMWHA which is not decreased in the KO mice and where the angiogenic potential of MM cells was decreased upon HA-depletion.

Taken together, we introduced novel in vitro and in vivo models with modulated HA content to investigate the impact of stromal HA on tumor cell behavior showing putative effects on proliferation and angiogenesis.

Literature: Amatangelo, M.D. et al. (2005). Am. J. Pathol. 167, 475-488.

Auvinen, P. et al. (2000). Am. J. Pathol. 156(2), 529-36.

Sapudom, J. et al. (2017). Acta Biomater. 50, 259-270.

Willenberg, A., et al. (2012). J. Invest. Dermatol. 132(2), 385-393.

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P192 | YB-1 expression and serine102 phosphorylation regulate tumorigenicity and invasiveness of melanoma cells by influencing epithelial-to-mesenchymal transition

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Cutaneous melanoma represents one of the most aggressive human tumour entities possessing a high tendency to metastasize. Cancer cells frequently exploit a highly conserved developmental programme, the epithelial-to-mesenchymal transition (EMT), to gain migratory and invasive properties promoting their metastatic spread. Cytoplasmic localization of the oncogenic transcription and translation factor Y-box binding protein 1 (YB-1) is a powerful inducer of EMT in breast carcinoma cells. Interestingly, EMT-like processes have also been observed in cutaneous melanoma despite its neural crest origin. Here, we can show that increased expression of YB-1 negatively affects patient survival in malignant melanoma and promotes melanoma cell tumorigenicity both in vitro and in vivo. Intriguingly, this effect seems to be mainly mediated by cytoplasmic YB-1 which does not exhibit phosphorylation at serine102 (S102). Moreover, we can demonstrate that S102 unphosphorylated YB-1 enhances the migratory and invasive potential of human melanoma cells in two dimensional and three-dimensional culture systems and facilitates acquisition of a mesenchymal-like invasive phenotype in the chick embryo model. Collectively, our data suggest, that the cytoplasmic activity of YB-1 stimulates tumorigenicity and metastatic potential of melanoma cells by promoting EMT-like properties.

P193 | von Willebrand factor-mediated platelet aggregates promote local disruption of the blood brain barrier in brain metastasis

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Venous thromboembolism is a frequent complication in melanoma patients with brain metastases and it has been associated

with high risk of metastasis. Prior to metastasize a tumor cell must interact with endothelial cells (ECs). We showed that melanoma cells activate human umbilical vein ECs (HUVECs) inducing the exocytosis of von Willebrand Factor (VWF). Shear stress in the blood causes VWF to unroll and form luminal fibers, which mediate platelet adhesion and thrombotic vessel occlusion. Based on a mouse model of lung metastasis we identified hallmarks of cancer-associated thrombosis, including VWF fibers and platelet aggregates. Interestingly, lung metastasis was enhanced in ADAMTS13 (a disintegrin-like and metalloprotease with thrombospondin type I repeats 13) deficient mice, characterized by prolonged intraluminal VWF fiber lifetimes. In contrast, blocking EC activation with the low molecular weight heparin Tinzaparin reduced the formation of VWF fibers and decreased the number of lung metastatic foci. These results demonstrated the contribution of luminal VWF in cancer-associated hypercoagulation and suggested the involvement of VWF in the formation of metastatic lesions.

However, in brain metastasis the role of VWF has not been depicted so far. We postulated that in brain vasculature melanoma-derived factors induce the release of VWF and promote the formation of a local platelet aggregations, which in turn disrupts the integrity of the Blood brain barrier (BBB) and facilitates extravasation of tumor cells. Our in vitro results showed that the expression of VWF in human brain microvascular ECs (HBMECs) is significantly lower compared to HUVECs. In line with this, in vivo analysis showed the presence of VWF in mouse brain vessels but in lesser extent compared to other organs. Using the ret transgenic mouse model, characterized by spontaneous development of brain metastases, we confirmed the formation of luminal VWF networks colocalizing with platelet aggregates in brain metastasis. However low expression of VWF in brain endothelium suggested us the involvement of platelet-derived VWF (PLT-VWF) in these events. Thus, we quantified the activation of platelet GPIIb-IIIa integrin complex, related with the extrusion of PLT-VWF, in ret brains. Our results showed a significant increase of activated platelets in metastatic brains. Next, we investigated the contribution of platelets disrupting the integrity of the BBB. We identified the secretion of vascular endothelial growth factor (VEGF) and Matrix Metalloproteinase 9 (MMP 9) in platelet releasates upon incubation with Ret cell supernatant. Using a combined impedance measurement system, we showed that platelet relasates increase the permeability of a HBMEC monolayer. Transmigration assays also confirmed the involvement of platelets mediating Ret cells transmigration. Surprisingly pre-treatment of platelets with Tinzaparin inhibited the secretion of permeability factors, reduced the effect of platelet releasates on permeability and decreased the transmigration of Ret cells.

In conclusion, our data provide new insights about the contribution of platelets in cancer-associated hypercoagulation and their contribution in the disruption of the BBB. In addition, our preliminary results suggest the potential of Tinzaparin as platelet inhibitor.

P194 | The combination of PI3Ki and MEKi: a treatment option for melanoma patients with either mutated or wildtype BRAF?

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New therapy concepts, such as immunotherapy with antibodies against CTLA4, PD-1 and PDL-1 as well as targeted therapy with BRAF and MEK inhibitors, have significantly improved the overall survival of melanoma patients in recent years. However, about 20% of patients do not respond to initial targeted therapy and at the same time, most of the tumours develop resistance through long-term therapy, e. g. by intensifying the activation of the PI3K-AKT-mTOR signalling pathway. Clinical studies in breast cancer show that both the pan-PI3K inhibitor BKM120 and the PI3Kα-selective inhibitor BYL719 have antitumour activity. This raises the question of whether these inhibitors are also a therapeutic option for melanoma and whether a combination with MEK inhibitors could further restrict growth and prevent possible development of resistance. At the same time, it will be investigated which mutations of the tumour indicate a positive therapeutic result.

BRAF and NRAS mutant cell lines, as well as directly isolated patient tumour cells are treated with the PI3K inhibitors BKM120 and BYL719 and in combination with the MEK inhibitor trametinib. In addition to the investigation of cell cytotoxicity and cell cycle remainder, the altered signal transmission is detected. Furthermore, the patient cells are sequenced in order to identify mutations that promote a positive therapeutic response. While the pan-PI3K inhibitor BKM120 is cytotoxic in almost all cell lines and patient cells, the PI3Kα-selective inhibitor BYL719 has only a limited antitumour effect. However, the combination of the PI3K inhibitors with the MEK inhibitor already shows regardless to their mutational status a significantly stronger cytotoxic effect at lower concentrations compared to monotherapies. Moreover, via the CAM-Assay the combination treatment of BYL719 and trametinib shows also in vivo a distinct tumour load reduction and the prevention of metastasis. These data show that the combination of PI3K inhibitors with MEK inhibitors could be a new therapeutic option for melanoma patients. By using PI3Kα-selective inhibitors, potential side effects could be reduced compared to pan-PI3K inhibitors.

P195 | Interaction of neutrophils and melanoma in the context of MAP kinase inhibition

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Patient survival in metastatic BRAFV600 mutant melanoma can be greatly improved by targeting the MAP kinase-signaling pathway. However, despite impressive and frequent responses, almost all tumors become resistant to targeted therapy. Various mechanisms of resistance have been described. Data from murine models indicate that myeloid cells can mediate resistance in targeted melanoma therapy. These data underline the complex interplay of immune cells with tumors which can promote either tumor regression or tumor cell survival. Previous data show that neutrophils are associated with poor prognosis and exhibit pro- as well as antitumoral characteristics. In this study, we investigated the interaction of neutrophils with melanoma cells under the influence of vemurafenib (a selective BRAF inhibitor) combined with cobimetinib, a MEK1/2 inhibitor. CFSE-labeled melanoma cells were co-cultured with human neutrophils in the presence or absence of the inhibitors. The viability of the melanoma cells was determined by flow cytometry by Annexin V/7-AAD staining. Furthermore, trans-well experiments with melanoma cells and neutrophils were performed to determine the role of cell-cell contact and the effect of soluble mediators. In blockade and rescue experiments, further insights could be achieved to understand the cell interaction and the effectiveness of the targeted therapy.

This study shows a discrete and direct survival promoting ability of neutrophils on melanoma cells in vitro. In addition, the induction of apoptosis in melanoma cells by combined MAPK inhibition is reversed by neutrophils. Our preliminary data suggest a new mechanism of resistance to MAP kinase inhibitors mediated by neutrophils. Elucidation of this unknown mechanism could lead to the development of therapeutic strategies to inhibit the resistance in melanoma patients to existing therapies.

P196 (OP04/04) | Melanoma in the humanized mouse: What influence has the macrophage phenotype on tumor growth?

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Question: Tumor cells can escape the patient's immune system by inducing immune suppression in the tumor microenvironment. To investigate the relevance of tumor associated macrophages (TAM) mediating suppression in human melanoma we established a melanoma model in the humanized mouse in order to study the complex interactions of human cancer and immune cells in vivo.

Our aim is to reprogram tolerance inducing TAM (M2) to immunostimulatory M1 macrophages or to eliminate them completely by using siRNA or small molecules like CSF-1R inhibitor BLZ945 and Tasquinimod which have been shown to influence the phenotype of TAM in different tumor models. Another approach is the use of a novel CuII based complex or Doxorubicin which block DNA synthesis. Those compounds are employed as free substances and bound to or encapsulated in nano sized carriers with the aim for a future systemic therapeutic approach with reduced side effects.

Methods: We perform in vitro cultures of human monocyte-derived macrophages to check transfection efficacy and toxicity of compounds. In vivo studies are carried out in a subcutaneous melanoma model of human melanoma cell lines in humanized NOD/SCID mice transgenic for HLA A2. After monitoring the tumor size up to eight weeks, we determine the composition of the tumor environment ex vivo via flow cytometry and IHC staining. Cationic polymer-based nanoparticles are used as siRNA-carriers and dendritic mesoporous silica nanoparticles and liposomes as drug carriers in vitro and in vivo.

Results: In vitro, the novel CuII based complex displays unexpectedly higher toxicity towards human macrophages, non dividing cells, than to several human melanoma cell lines. This is in contrast to Doxorubicin, which is more toxic towards melanoma cell lines. The release of encapsulated CuII complex and Doxorubicin is time dependent and results in an overall lower toxicity compared to the equivalent dose of free substance. This indicates a functional gater system. We measured cell viability via Resazurin assay and accumulation of doxorubicin in the nuclei by confocal microscopy.

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

In vivo, 2 out of 3 used melanoma cell lines do grow in NOD SCID mice. We are able to retrieve human immune cells up to four weeks after their transfer in the tumor vicinity and analyze PBMC subpopulations and their phenotype. Spleen and tumor showed a distinct composition of immune cell infiltration in immunohistochemistry staining.

Conclusion: Our mouse model is suitable to investigate the influence of small molecules and siRNA on cells in the tumor microenvironment and resultant effects on tumor growth in a fully human setting. We are currently performing therapeutic in vivo experiments in our model.

P197 (OP03/03) | Crosstalk between cytokine-induced apoptosis and cytokine-induced senescence in human cancer cells

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In recent years, clinical studies showed that targeted cytotoxic therapies with Braf inhibitors led to a dramatic reduction of the

tumor load in patients with melanoma metastases whereas their overall survival rate was only slightly increased. In contrast, the newly introduced immune check-point inhibitors showed a different clinical course: after a first regression phase of the tumors the lesions partially remained constant. As a result of the therapy, the overall survival rate was clearly increased, and many patients live with a detectable residual tumor load. Thus, immune checkpoint inhibitors seem to activate two different antitumor mechanisms. During the regression phase, the biologicals induce apoptosis by reactivating cytotoxic T cells, whereas in the consolidation phase the therapy leads to non-toxic, cytokine-dependent immune surveillance of the residual tumors or metastases. We have demonstrated previously that the Th1 cytokines interferon-gamma (IFNgamma) and tumor necrosis factor (TNF) can control tumors by induction of cellular senescence in the absence of overt tumor cell death. Here, we investigate whether the cytokine cocktail either induces apoptosis or senescence, and how the decision of the tumor cells is regulated. For this, apoptosis-resistant, caspase-3-deficient, control vector-transfected MCF-7 breast cancer cells or apoptosis sensitive, caspase-3-transfected MCF-7 cells were treated for different intervals with doxorubicin or a cytokine cocktail consisting of IFN-gamma plus TNF. We then determined cell viability by trypan blue exclusion and by measurement of the mitochondrial activity using the XTT test. The cell cycle activity of the cancer cells was further analyzed by a specifically designed growth assay, with a consecutive drug or cytokine treatment and removal phase. Additionally, senescence induction was measured by the senescence-associated (SA)-beta-galactosidase assay. First results showed that doxorubicin and IFN-gamma plus TNF exerted low toxic pressure in caspase-3-deficient MCF-7 cells but instead permanently inhibited cell cycling, induced the flat, egg-shaped senescent morphology and increased SA-betagalactosidase activity. On the other hand, caspase-3-transfected MCF-7 cells were more sensitive to doxorubicin- or cytokine-induced cell death. After challenge with the drug or the cytokines, the cells clearly showed the round and shrunken morphology of apoptotic cells, including membrane blebbing. In addition, there was no induction of SA-beta-galactosidase in caspase-3-transfected MCF-7 cells. Growth assays further revealed that caspase-3-deficient MCF-7 cells remained growth arrested but did not die after drug removal whereas the number of living cells in caspase-3- transfected MCF-7 cells steadily declined. However, at lower, senescence-inducing drug concentrations the caspase-3-expressing MCF-7 cells already restarted growing at earlier time points as compared with their caspase-free counterparts.

Taken together, our data show that apoptosis and cellular senescence are two different cellular conditions that can be activated by the same agents or signals, presumably in parallel. In case of a severe disruption of the apoptosis signaling cascade, as for example by loss of the executioner caspase-3, cellular senescence may then act as a non-toxic backup to control malignant cancer growth.

P198 | Stat1-dependent induction of cancer cell senescence via Th1 driven immunotherapy in advanced cancer

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Immunotherapy with tumor-associated antigen (TAA)-specific T-helper-1 (Th1) cells mediates anti-tumor effects in patients with skin cancers, such as melanoma or squamous cell carcinoma. Similarly, adoptive transfer of TAA-specific Th1 cells prolongs the life of transgenic tumor-bearing mice by induction tumor cell senescence. Immune checkpoint inhibitors-based therapy (ICI) with monoclonal antibodies against exhaustion-associated surface molecules reactivated T cells and may induce durable therapeutic stability in a variety of metastatic cancers. While cancer regression has been described, the mechanisms causing the long-lasting tumor dormancy remain unknown. To investigate the mechanism underlying cancer cell dormancy we treated mice with T antigen (Tag)-expressing cancers with TAA specific Th1 cells. Th1 cells prolonged the life time of transgenic tumor-bearing mice by induction of tumor cell senescence. Combining adoptive transfer of Th1 cells with ICI increased life time significantly. To investigate the mechanisms of this combined therapy we treated Tag2 mice having advanced cancers, 4 weeks prior to cancer induced death with adoptive transfer of TAA-specific Th1 cells and PD-L1/LAG-3 (Programmed-Death-Ligand-1/Lymphocyte-Activation Gene 3). The therapy restored a normal health status in the mice, destroyed the large cancers partly and induced a p16INK4a-positive and Ki67-negative senescent phenotype in the remaining cancer cells. The therapeutic effect was strictly dependent on an intact interferon (IFN)/Stat1-signaling pathway in the cancer cells. Tag2 driven cancers of Stat1-knockout mice did not respond to the therapy. To confirm this mechanism we inoculated Tag driven cancers subcutaneously and treated the advanced cancers with PD-L1/LAG-3. We compared the tumor growth and blood glucose development of mice treated with ICI and compared them to isotype treated mice. Preliminary data from CD8-depleted mice showed that treatment with ICI controlled exclusively Stat1-competent cancers but not Stat1-deficient cancers. Our work suggests that Stat1-signaling contributes to cancer control by ICI, possibly through the induction of cytokine-induced senescence in cancer cells.

P199 | A dual role of neutrophils in cutaneous melanoma

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Neutrophils exert dual roles in cancer progression due to their high phenotype plasticity, which is strongly context dependent and governed by the local niche. In a syngeneic mouse melanoma model overexpressing CXCL5, we identified functionally different neutrophil subsets in the primary tumor and the metastatic niche. Primary tumor associated neutrophils acquired a pro-tumorigenic phenotype, characterized by high PD-L1, CXCR4 and CCR5, and revealed immunosuppressive actions in the context of induced adaptive immunity. Contrary, residential neutrophils in the premetastatic lung revealed a different phenotype, are activated by melanoma-derived CXCL5 and protect against the development of lung metastases, likely in a ROS dependent manner. Our findings uncover a microenvironment-dependent control of neutrophil phenotypes to exert either pro-tumorigenic or anti-tumorigenic functions seen in many tumor models. In addition, it may explain why some patients do not respond to immunotherapy with checkpoint inhibitors, and provide novel therapeutic avenues to prevent metastatic spread of melanoma.

P200 | Tanshinone IIA a promising approach in Merkel cell carcinoma?

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Introduction: Merkel cell carcinoma (MCC) is a rare, aggressive skin cancer with rising incidences over the past years. Chemotherapy has been standard of care in the metastatic disease, despite, low durable response rates and significant toxicities. Therefore, the recent progress with immune checkpoint inhibitor is linked to great expectations. Nevertheless there is a strong need for new agents in the treatment of MCC. Tanshinone IIA (TAII), a natural diterpene extracted from salvia miltiorrhiza is an interesting molecule. In this project we investigated the anti-tumorigenic effects of TAII in merkel cell carcinoma.

Methods: Merkel cell carcinoma cell lines MKL-1 and MCC-13 were cultured in vitro and treated with or without TAII. Effects of TAII on proliferation and cell cycle were analysed by BrdU assay and flow cytometry. Apoptosis was measured by quantifying mono-and olico-nucleosomes, measuring caspase-3/7, -8, -9 activity assay and immunoblotting. Cytochrome C release assay was performed.

Results: TAII inhibits significantly cell growth in a concentration dependent manner without major cytotoxicity. Cell cycle analysis revealed an elevation in Sub-G0 after TAII treatment pointing to apoptosis. Consequently we demonstrated, that TAII treatment leads to a significant induction of apoptosis measured by cell death detection ELISA and to an upregulation of caspases 3, 7, 8 and 9. Additional cytochrome c is released from mitochondria and pro-apoptotic proteins from the bcl-2 family are upregulated. First evidence is pointing to a JNK and p53 depending signalling mechanism.

Conclusions: Our results demonstrate pro-apoptotic and anti-proliferative effects of TAII in merkel cell carcinoma. Therefore, it could be speculated that TAII may provide a novel therapeutic option in this tumour entity. Further investigations are necessary to evaluate the specific mechanisms resulting in the observed changes.

P201 | Chitinase-3-like 1 modulates extracellular matrix and release cytokines

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Chitinases are highly conserved enzymes ubiquitously present in bacteria, fungi, plants and mammalians. The mammalian chitinase(-like) proteins comprise nine members. In humans, they have been attributed to inflammatory diseases such as arteriosclerosis, or to tumor progression. In our current study, we have investigated the molecular function of the mammalian chitinase-3 like 1 (CHI3L1). Although CHI3L1 was shown to induce angiogenesis and cell migration, the molecular mechanisms involved are still unclear. Two hypotheses have been put forward, namely translocation of CHI3L1 from the cytoplasm into the cell nucleus affecting gene transcription, or CHI3L1-induced remodeling of the extracellular matrix.

Because clinical data suggest a correlation between CHI3L1 plasma levels and the severity of melanoma, we first tested the impact of recombinant CHI3L1 on the migratory properties of human (BLM) and murine (Ret) melanoma cells using a transwell assay. In agreement with previous reports, we found that increased levels of CHI3L1 contribute to an increased cell migration. Further experiments documented that overexpression of human CHI3L1 in BLM cells boosted the levels of cytokines and growth factors (e.g. CCL-2 and VEGF-A) in the cell supernatants. However, we were not able to measure elevated expression levels of corresponding genes by quantitative real time PCR. Moreover, neither tracking of red-fluorescence protein tagged CHI3L1 by live cell fluorescence microscopy nor immune fluorescence staining could confirm the previously postulated nuclear localization of CHI3L1.

Accordingly, we have followed the second hypothesis, suggesting a potential remodeling of the extracellular matrix. Using fluorescence spectroscopy, we have analyzed the binding of CHI3L1 to heparin and other glycosaminoglycans (GAGs). A dissociation constant of 60 μM indicates a physiologically relevant binding affinity of CHI3L1 to heparin. Immune histochemical analysis of human melanoma tissue sections documents a predominant extracellular occurrence of CHI3L1 suggesting an interaction with GAGs of the extracellular matrix. Next, we analyzed whether CHI3L1 is able to remodel the extracellular matrix through the release

of GAG stored factors such as VEGF-A or CCL-2. To this end, we developed an ELISA based ligand binding assay. We found that physiological concentrations of CHI3L1 of about 100 ng/mL were sufficient to release GAG-bound VEGF-A or CCL-2. In comparison to CCL-2, the competition of CHI3L1 and VEGF-A was significantly more pronounced. Tube formation assays further confirmed that CHI3L1 promotes angiogenesis through the release of VEGF-A from the matrix.

In conclusion, we found that CHI3L1 modulates the availability of extracellular matrix retained signaling molecules such as CCL-2 or VEGF-A through competitive binding to GAGs. Although further functional in vivo experiments are required to confirm the molecular impact of our findings, we postulate that blockage of CHI3L1 may reduce tumor-induced angiogenesis and tumor cell migration

P202 | Active recruitment of mast cells to the microenvironment of malignant melanoma

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Background: Malignant melanoma is the most aggressive type of skin cancer, with a five-year overall survival rate of <15 percent in untreated patients. Histologically, melanomas are characterized by an inflammatory infiltrate, which also contains numerous mast cells (MC). However, the role of MC in the microenvironment of melanoma remains poorly understood. In the present study, we aimed to investigate the number and distribution of MC in melanomas and to analyze recruitment of MC by melanoma cells.

Methods: Distribution, number and degranulation of MC were assessed in skin sections of primary melanomas (n=31) and melanoma metastases (n=10) using immunohistochemistry. Melanoma sections were compared to cutaneous sections of healthy subjects (n=7) and atopic dermatitis (n=11) as controls. Migration of a human MC line (HMC-1) towards two melanoma cells lines (MaMel21, MaMel63a) was examined using a Boyden chamber assay and the real-time migration system xCELLigence®. Migration towards PBS and a keratinocyte cell line (HaCaT) served as controls.

Results: Numbers of tryptase-positive MC were significantly increased in the periphery of primary melanomas and reduced in melanoma metastases compared to normal skin. Typically, MC were found to align along the rim of the inflammatory infiltrate of the tumor periphery. The number of MC in the tumor periphery corresponded well to MC counts in sections of atopic dermatitis. When we analyzed degranulation of MC in different skin sections, we observed more degranulated and less not-degranulated mast cells in the tumor center of melanomas and melanoma metastases compared to normal skin, tumor periphery and atopic dermatitis. Migration of HMC-1 cells was significantly increased (10-fold) towards the melanoma cell lines compared to PBS and HaCaT cells. Comparing the two melanoma lines, MaMel21 induced even more pronounced migration than MaMel63a. Assessing the time course of migration using the xCELLigence® system, we found that most HMC-1 cells migrated towards melanoma cells within the first 70 minutes.

Conclusion: Taken together, we found increased numbers of MC and enhanced MC degranulation in primary melanomas and melanoma metastasis. Furthermore, we observed pronounced and fast migration of a MC line towards melanoma cells lines. These results suggest an active role of MC in the microenvironment of malignant melanoma.

P203 (OP06/01) | Targeting cell cycle phase-specific drug sensitivity for melanoma therapy

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The development of small molecule MAPK pathway inhibitors (MAPKi) and antagonists of the immune checkpoints has revolutionized melanoma therapy. However, MAPKi only work in approximately 40% of cases as a BRAFV600 mutation must be present; moreover, rapid development of resistance is common. Immune checkpoint inhibitors (ICi) show response rates up to 60%, depending on drug or combination, with durable effects but resistance can still occur. Thus there is a clear need to develop combination therapies to delay the onset of resistance.

Many anti-cancer drugs impact the cell cycle but are also dependent on specific cell cycle phases, which results in cell cycle phase-specific drug insensitivity. The tumor microenvironment is characterized by cancer cell subpopulations with heterogeneous cell cycle profiles. For example, hypoxic tumor zones contain clusters of cancer cells that arrest in G1-phase whereas actively cycling cells cluster around blood vessels. Importantly, neoplastic cells exhibit differential drug sensitivity based on their residence in specific cell cycle phases.

We have established a model to study the effects of the cell cycle on drug sensitivity in real-time: Utilizing two- and three-dimensional melanoma culture models in combination with fluorescent cell cycle indicators (FUCCI), we investigated the effect of G1-arrest on drug sensitivity. G1-arrested melanoma cells were more resistant to apoptosis induced by agents that selectively target S/G2/M phase cells, such as the proteasome inhibitor bortezomib or the alkylating agent temozolomide. In contrast, G1-arrested cells were more sensitive to MAPKi-induced cell death as this pathway is essential for G1-phase progression. Of major clinical relevance, pretreatment of melanoma cells with sub-lethal but G1-arresting doses of a MAPKi resulted in resistance to temozolomide or bortezomib. We also found that changing environmental conditions, such as applying hypoxia, resulted in protective G1- arrest. However, hypoxia can also result in endoplasmic

reticulum (ER) stress. If the level of ER stress is persistent or excessive it switches to an apoptotic response. We show that the protective effect of G1-arrest can be overridden by the apoptotic effect of ER stress. This was then used as a therapeutic approach: Using the F-XBP1ΔDBD-venus reporter construct, to visualize ER stress, we showed that single agent low dose ER stress-inducing agents, bortezomib and fenretinide, induced ER-stress and cell cycle arrest but only limited cell death, while the combination resulted in synergistic apoptosis. Taken together, our data demonstrate that cell cycle-tailored targeting of metastatic melanoma can improve therapy outcomes and that additional induction of ER stress is a strategy worth investigating to further improve melanoma therapy. Furthermore, we propose that our real-time cell cycle imaging 3D melanoma spheroid model could be utilized as a tool to measure and design drug scheduling approaches.

P204 | NRAS and BRAF-induced senescence in primary human melanocytes

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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 tumour 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, vacuolization and an oval to round cell shape and an increased activity of the senescence marker ß-galactosidase.

In our current study we deciphered differences of BRAF versus NRAS-induced senescence in primary human melanocytes. We found that oncogenic NRAS induces a more potent senescence response in primary human melanocytes. In particular senescence features such as formation of senescence-associated heterochromatin foci, ß-galactosidase activity and morphological changes were more prominent in NRAS compared to BRAF expressing melanocytes. We currently decipher underlying signalling events that a responsible for the distinct phenotypes.

P205 | Combined action of histone deacetylase inhibition and interferon gamma drives cancer cells into senescence.

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Combined action of T-helper-1-cell cytokines interferon gamma (IFN-γ) and tumor necrosis factor (TNF) drives cancer cells into a state of cell-cycle arrest, termed cellular senescence. However, systemic treatment with TNF is curbed by significant toxicities and necessitates the search for different nontoxic agents for combination with IFN-γ in vivo.

Here, we show that the pan-histone deacetylase inhibitor vorinostat and IFN-γ synergize and induce cellular senescence in human and murine tumor cells in vitro. Combined therapy of human A-204 rhabdomyosarcoma and MCF-7 breast cancer cell lines induced robust growth arrest even after withdrawal of both drugs. Combination treatment induced a marked reduction of the S phase fraction and increased levels of senescence associated beta-galactosidase. Moreover, vorinostat led to downregulation of DNA methyltransferase 1 (DNMT1) as determined by western blot. In this line, pharmacological inhibition of DNMT1 with 5-Azacytidine (5-azaC) sensitized A204 cells towards treatment with IFN-γ.

In further experiments, we used insulinoma cells from an oncogenic mouse strain in which the Simian virus 40 large T antigen (Tag) expressed under the control of the rat insulin promoter creates tumors by attenuating p53- and Rb-mediated cell cycle control. First data revealed that vorinostat and IFN-γ also induced a senescent phenotype in insulinoma cells. Whether this senescence induction requires the cell cycle regulator p16INK4A and the signal transduction proteins TNFR1 or STAT1 will be investigated by targeted knock-out of these factors in insulinoma cells.

In conclusion, our data demonstrate that histone deacetylase inhibition leads to downregulation of DNMT1 and sensitizes tumor cells towards IFN-γ. Combined vorinostat and IFN-γ induces cellular senescence and may be a therapeutic option in vivo.

P206 | Inhibition of melanoma cell derived prostaglandin E2 amplifies T-cell receptor signalling and shows clinical efficacy in combination with anti-PD1 therapy

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Aim: We wanted to investigate whether melanoma cells actively secrete factors that impair T-cell receptor signaling on T-cells.

Methods: With the help of a luciferase based T-cell reporter cell line we analyzed the effects of melanoma cells and secreted factors on T cell receptor signalling. By ELISA and mass spectrometry we identified and quantified prostaglandin E2 (PGE2). Aspirin and celecoxib were used as cyclooxygenase inhibitors to treat melanoma cell lines and short-term cultured patient derived metastatic melanoma cells in order to prevent PGE2 synthesis.

Results: We could show that melanoma cell supernatants actively suppressed IL2-promoter activity after stimulation of the TCR. We further identified the hormone-like lipid PGE2 as a main mediator of this effect. PGE2 was secreted in effective concentrations from several melanoma cell lines and short-term cultured patient derived melanoma cells. COX-2 inhibition using either aspirin or celecoxib not only abolished PGE2 secretion but also significantly diminished the suppressive effect of melanoma conditioned medium on T cells. In line with the work of Zelenay et al. two patients with moderate progressive disease under anti-PD-1 therapy shifted into long lasting regressions after the addition of aspirin as shown by PET/CT scanning.

Conclusion: We therefore hypothesize, that human metastatic melanomas use a cyclooxygenase dependent evasion of immunity that could be tackled by the concerted action of COX and checkpoint inhibitors.

P207 | Ultraviolet (UV)-A irradiation induces prolonged changes in metabolism which influence, via enhanced lactic acid production, melanoma invasion in vitro

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Melanoma is a malignant tumor with high mortality for which exposure to ultraviolet (UV) A radiation is considered to be a critical risk factor. Especially UVA (320-400 nm) radiation induces the formation of reactive oxygen species (ROS) which damage cellular molecules. It was recently shown that UVA radiation can induce murine melanoma, but the role of UVA in the progression of melanoma is not clear. This is important as many initial melanomas are not immediately recognized and patients often continue sun exposure. During early progression of melanomas before metastasizing, most melanomas show initial proliferation of melanoma cells and a metabolic characteristic of most proliferating tumor cells are high rates of aerobic glycolysis.

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

Upon UVA radiation, initial melanoma cells show increased glucose consumption and increased lactic acid production. With in vitro invasion assays we show, that lactic acid, which can be produced via UVA irradiation, increases invasiveness of initial melanoma cells in in vitro invasion assays. This effect is partially mediated by ROS which are induced by UVA radiation, as treatment with ROS scavengers ameliorates UVA induced lactate production and invasion. Interestingly UVA induced glucose consumption and lactate production are present up to 5 days after cessation of UVA irradiation, indicating a prolonged change of glucose metabolism. Furthermore expression and secretion of tumor relevant matrix metalloproteinases (MMP) and urokinase plasminogen activator (uPA)are highly upregulated upon treatment with lactic acid. Therefore we could show in melanoma cells, derived from melanomas of early progression, that production of lactic acid, induced by UVA radiation, increases in vitro invasiveness of initial melanoma cells via expression of MMPs and uPA.

P208 | Cancer-associated immune suppression in cutaneous T cell lymphoma

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In cutaneous T cell lymphoma (CTCL), the malignant T cells are a source of suppressive Th2 cytokines, such as IL-4, and progressive impairment of cellular immunity is a hallmark of the disease. IL-4 is known for its capacity to sustain Th2 cell differentiation, when acting directly on T cells, but can also initiate an IL-12 dependent negative regulatory feedback loop and initiate protective Th1 immune response when present during the initial activation of dendritic cells (DC). Interestingly, we found an association of increased IL-4 production and, at the same time decreased IL-12 levels, with advanced stage CTCL. Neutralization of IL-4 restored Th1 but not Th17 immune responses in CTCL, and DC activation was directly suppressed through co-inhibitory T cell surface molecules. This points out towards an abrogated DC-T cell regulatory loop in patients with CTCL and suggests an immune escape mechanism that allows cancer cells to evade recognition from the innate immune system, and subsequently abrogate the differentiation of a protective non-malignant effector $CD4^+$ T cell population.

P209 | Phenotype switching as a resistance mechanism to BRAF and MEK inhibition

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Targeted therapy in melanoma has had great success in extending progression free survival for melanoma patients. These include BRAF inhibitors for patients with a BRAF V600 mutation and MEK inhibitors for patients with an NRAS G12 or Q61 mutation. To elucidate the genetic and non-genetic mechanisms of resistance, we have selected 31 BRAF mutated and 23 NRAS mutated melanoma cell cultures from the Melanoma Biobank at the Dermatology Clinic of University Hospital Zurich for RNAseq and targeted panel

sequencing. We have in vitro test all 31 BRAF mutated cell cultures for sensitivity to encorafenib and 15 of the cell cultures were resistant. We have in vitro tested all 26 NRAS mutated melanoma cell cultures for binimetinib resistance and found that 11 are resistant. Principal component analysis of RNAseq data show clustering of the sensitive and resistant cell cultures. Differential gene expression analysis between the sensitive and resistant cell cultures show genes involved in phenotype switching, such as MITF, TYR, WNTA, and AXL. This suggests that phenotype switching could be a general mechanism responsible for sensitivity to targeted therapy inhibitors in the BRAF and NRAS mutated populations. Targeted panel sequencing of the sensitive and resistant cultures did not reveal any common genetic variants, however in the BRAF resistant population about 40% of the cell cultures contained an NRAS activating mutation. Most resistant cell cultures had their own specific mutations suggesting that genetic resistance mechanism could be patient specific. Overall, using a combination of RNAseq and targeted panel sequencing, potential genes and mutations could be discovered as biomarkers for sensitivity to BRAF and MEK inhibitors.

P210 | CXCL13 and BOB1 expression in initial biopsies of mycosis fungoides with stable early stage and later tumor stage disease

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Introduction & Objectives: Mycosis fungoides (MF) is the most common cutaneous T-cell- lymphoma (CTCL) accounting for approximately 50% of all CTCL with a wide range of initial disease presentation and evolution over time. However, in early disease stages biomarkers as indicators of a later progression have not yet been identified.

Materials & Methods: Of 173 patients with confirmed MF, treated at the Department of Dermatology, University Kiel, (1995-2015), 17 patients with stable disease MF ("MF stable") defined as T1aN0M0B0 over a period of more than 5 years were identified and compared to 20 patients with later evolution to tumor-stage MF ("MF tumor"). We investigated the initial diagnostic specimen of these patients by gene expression profiling for 770 different genes related to immunological mechanisms and cancer (Nanostring/nCounter) with a protocol optimized for formalin fixed paraffin embedded tissue. For the validation of differential gene expression, immunohistochemistry was performed for selected markers, i.e. CXCL13 and BOB1.

Results: The gene expression profiling identified 36 genes with a statistically significant differential expression between "MF stable" and "MF tumor" specimen ($P \leq .05$). Within these, we observed a higher expression of several genes pointing towards a follicular T-helper phenotype in samples of patients with later tumor-stage development, among them CXCL13 and IL21. Additionally, in these samples higher B-cell marker gene expression was detected (CD79A). Semiquantitative analysis of immunohistochemistry for BOB1 and CXCL13 in fact confirmed a higher number of positive cells in lesions of "MF tumor" compared to "MF stable".

Conclusions: We identified genes in initial biopsies of MF that differ between diseases with a long stable course and those with progression to a tumor stage. Interestingly, differential gene expression in samples of patients with later progression points towards a follicular T-helper cell phenotype. For Bob1, which is basically a B-cell specific transcriptional coactivator, a role in the memory function of $CD4^+$ cells has been described recently. Further exploration is required to determine, whether higher expression in early disease stages might be a relevant contributive factor to tumor development in MF patients. The present data provides insights into pathogenesis and might display future perspectives for routine diagnostic biomarkers.

P211 | Impact of oncogenic BrafV600 on melanoma pathogenesis in Hgf-Cdk4R24C mice

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Genetically engineered mice have successfully been used to model melanoma pathogenesis and to study the reciprocal interactions between tumor and immune cells in the microenvironment. In our previous work we found that melanocyte specific tamoxifen-induced activation of oncogenic BrafV600 followed by a single application of the carcinogen dimethylbenzanthracene (DMBA) in mice carrying the R24C germline mutation in the Cdk4 gene (BrafV600E-Cdk4R24C mice) leads to the simultaneous development of both immune-cell poor pigmented as well as immune-cell rich amelanotic melanomas. Here we investigated how conditional activation of oncogenic BrafV600 affects melanoma pathogenesis in Hgf-Cdk4R24C mice (HC mice). Constitutive transgenic overexpression of the melanocyte growth factor HGF leads to increased numbers of skin melanocytes and predisposes mice to the development of primary cutaneous melanomas which spontaneously metastasize in lymph nodes and lungs. We generated BrafV600E-Hgf-Cdk4R24C mice (B-HC mice) by crossing mice carrying the conditionally activatable oncogenic BRAFV600E knockin allele and the tamoxifen-inducible cre recombinase under the control of the melanocyte-specific tyrosinase promotor (Tyr::CreERT2-LSLBrafV600E) with HC mice. 6-8 weeks old B-HC mice were treated with 33 μg of 4-hydroxytamoxifen (4OHT) on three consecutive days on the shaved back followed by a single dose of 25 μg DMBA on day 4. Melanoma growth was investigated over time. HC mice treated with 25 μg DMBA only served as controls. Activation of oncogenic BrafV600E with 4OHT led to a significantly earlier onset and accelerated growth kinetics of DMBA-induced skin melanomas in B-HC mice when compared to DMBA induced melanomas in HC mice. B-HC mice had to be sacrificed after 58 days (3) whereas HC

mice lived 117 days (7) after tumor induction. Importantly, B-HC mice developed both immune cell poor melanotic as well as immune-cell rich amelanotic melanomas with morphological features of malignant peripheral nerve sheath tumors. Taken together, activation of oncogenic BrafV600 has similar effects on melanoma development in Hgf-Cdk4R24C and Cdk4R24C mice. In ongoing experiments we are further investigating the impact of oncogenic BrafV600 on the immune cell composition of the melanoma microenvironment.

P212 (OP01/06) | Role of 2A-DUB/Mysm1 in DNA damage responses in the skin and hematopoietic system

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Alterations in chromatin modifications and in chromatin-modifying enzymes have been identified in a variety of human malignancies including skin cancers and may have roles at different stages of tumorigenesis. The deubiquitinase 2A-DUB/Mysm1 catalyzing the removal of ubiquitin from lysine residue K119 of core histone H2A has mainly been implicated in transcriptional activation during hematopoietic development and to regulate stem cell and progenitor fate through interactions with the Arf-p53- Puma axis. Recently, novel roles of MYSM1 in melanoma, colon carcinoma, and other tumors could be demonstrated pointing to an interplay with growth factor signaling cascades and with the Cdkn2a-p53 tumor suppressor pathway during tumor formation.

Because insufficient DNA-repair is considered a major pathogenic factor in UV induced mutagenesis, skin cancer formation, as well as cancer cell genomic instability and epigenetic modifications are known to be critically involved in detection and repair of DNA lesions, we investigated the potential molecular role of 2A-DUB/Mysm1 in DNA-damages responses (DDR) upon cellular stress in an ultraviolet (UV-)irradiation model in the skin and upon aging and genotoxic stress using Mysm1- mutant mouse models and human tumor cell lines.

High MYSM1 expression was detectable in melanoma cell lines A375 and SKMEL- 28 as well as in HaCat and KG1a cells. Indicative of a role MYSM1 at different stages of melanoma formation increases in MelanA- and MYSM1-double-positive cells were found in nevi, superficial spreading melanoma (SSM), and melanoma metastases compared with normal human skin in context with frequent co-localization of MYSM1 with c-MET and PAX3. Upon 7,12-Dimethylbenz[a] anthracene(DMBA)- treatment and UVB irradiation, Mysm1 expression was significantly increased in different cell types of the skin in young adult mice. Consistent with a function of Mysm1 in the control of UV-induced DDR in the skin, γH2AX levels were significantly increased in basal cells of the epidermis. In addition formation rates of epithelial tumors and melanoma were evaluated upon long-term UVB-irradiation in Mysm1- deficient vs. wild-type C57BL/6 mice and correlated with immune cell infiltration and activation of cellular stress signaling.

Mechanistically, MYSM1 co-localized to sites of DNA-damage identified by DNA damage marker γH2AX in the nuclei upon induction of DNA double-strand breaks (DBS) with topoisomerase inhibitor etoposide in peripheral blood mononuclear cells (PMBCs) and A375 melanoma cells in vitro in immunofluorescent analyses. Contrarily, MYSM1 was absent in compacted chromatin regions detected by phospho-Histone H3 (pHH3) and Ki-67 and re-localized to the cytoplasm during M-phase of the cell cycle in synchronization experiments. Consistent with a role in promotion of tumor cell survival, MYSM1 expression was regulated by Ras/MEK/MAPK and AKT signaling and decreased upon treatment with MEK- und AKT inhibitors. In addition, expression of DNA damage marker γH2AX was reduced upon MEK/AKT dual inhibition in A375 cells, an effect that was aggravated upon shRNA mediated knockdown of MYSM1. Molecular interactions of MYSM1 with transcription factors and other epigenetic enzymes during DNA repair processes were further investigated via proximity ligation assay (PLA) and Co-IP in combination with mass spectrometry to examine the crosstalk with kinases such as ATM and ubiquitin ligases such as PCR1 component BMI1 and RNF2 and other enzymes.

In conclusion our current data indicate an involvement of MYSM1 in DNA damage responses in the skin with potential implications for skin tumor formation and in addition for therapeutic responses to inhibitors of MAPK and PIK3 signs

MISCELLANEOUS

P213 | Epidermal lipid composition, barrier integrity and eczematous inflammation impact skin microbiome composition

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Genomic approaches have revealed characteristic site-specificities of skin bacterial community structures. In addition, in children with atopic dermatitis (AD), characteristic shifts were described at creases and in particular during flares, which have been postulated to mirror the disturbed skin barrier function and/or cutaneous inflammation. However, is yet unclear whether altered cutaneous bacterial community structures in AD are restricted to predilection sites or are a general feature, and what impact distinct abnormalities of epidermal barrier function and eczematous affection have.

The skin microbiome was determined by bacterial 16S rRNA sequencing at 4 different body sites of 10 AD patients and 10 healthy controls matched for age, sex and FLG mutation status. In AD patients, in addition acute and chronic AD lesions were analysed. All sampling sites were characterized for skin physiology parameters including chromatography-based lipid profiles.

Epidermal lipid composition, in particular levels of long-chain unsaturated free fatty acids, strongly correlated with bacterial composition, in particular Propionibacteria and Corynebacteria abundance. AD displayed a distinct community structure with an increased abundance and altered composition of staphylococcal species across body sites with the strongest peculiarities seen on chronic lesions, and a progressive shift from nonlesional skin to acute and chronic lesions. FLG deficient skin showed a distinct microbiome composition partly resembling the AD-related pattern.

Stratum corneum integrity and epidermal lipid composition impact the skin microbiome composition. AD is characterized by shifts of the skin microbiome also at nonaffected sites, but in particular at sites of predilection and eczematous affection. Skin inflammation appears to overlay locoregional differences in microbiome composition.

P214 | The PPAR-γ modulator, N-acetyl-GED, as a novel therapeutic strategy for lichen planopilaris and other scarring alopecias

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In lichen planopilaris (LPP) patients, epithelial stem cells (eSCs) in the hair follicle (HF) bulge lose their immune privilege, are attacked by a cytotoxic $CD8^+$T-cells driven inflammatory response, and undergo epithelial-mesenchymal-transition (EMT). This results in scarring alopecia, for which fully satisfactory therapy remains to be developed. Since PPAR-γ agonists have been shown to inhibit EMT and have antiinflammatory properties, the current study aimed at investigating whether N-acetyl- GED (AGED), a topically applicable PPAR-γ modulator with a favourable toxicology profile, can protect or rescue human HFeSCs from experimentally induced EMT and reverse EMT in established disease ex vivo, i.e. in HFeSCs of scalp skin samples from LPP patients.

For this purpose, we first administered AGED (0.01, 0.1, and 1 mM) before or after EMT induction ex vivo by treating organ-cultured, full-length healthy human scalp anagen VI HFs with an EMT cocktail composed of epidermal growth factor, transforming growth factor-β1, interferon-γ, and peptide A, the selective E-cadherin blocking peptide. Our results showed that all tested concentrations of AGED (0.01, 0.1, and 1 mM), protected HFs from experimentally-induced EMT ex vivo, as indicated by the ability of AGED to significantly prevent the down-regulation of E-cadherin and the up-regulation of $vimentin^+$ and $SLUG^+$ cell number in the bulge of treated HFs ex vivo. Based on the analysis of classical EMT markers, AGED even partially rescued HFs from experimentally-induced EMT ex vivo by significantly decreasing the number of $vimentin^+$ or $SLUG^+$ cells, but only tendentially normalizing E-cadherin expression. Next, we have treated human scalp organ-cultured biopsies from LPP patients ex vivo with AGED (0.1 mM). Our data showed that AGED (0.1 mM) greatly reversed the EMT protein signature in the bulge of LPP affected HFs ex vivo, as indicated by significantly increased of E-cadherin expression and significant decreased of $vimentin^+$, $SLUG^+$ cell number. Importantly, AGED also increased the number of cytokeratin 15^+ cell number in the bulge of LPP affected HFs. In addition, the number of immune cells, namely $CD8^+$ T-cells (key effector cells in LPP) and MHC class II + cells, in and around the bulge of LPP affected HFs were decreased after AGED treatment.

Taken together, our data suggest that AGED deserves to be further clinically explored as therapeutic option LPP and other scarring alopecias.

P215 | A new human folliculoid assay for investigating neuroectodermal mesenchymal interactions in vitro

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In order to develop new hair care products and therapeutic agents for hair disorders, reliable and predictable preclinical models are needed. Since advanced techniques in plastic surgery and hair transplantation have reduced the availability of human hair follicles (HF) for organ culture assays, instructive and predictive "HF equivalent" models are needed that mimic the complexity of the neuroectodermalmesenchymal interaction between hair matrix keratinocytes, HF pigmentary unit (HFPU) melanocytes, and inductive dermal papilla fibroblasts whose interactions drive hair shaft production and pigmentation as well as HF cycling.

In the current study, we have developed a folliculoid assay in which plucked human anagen VI scalp hair shafts (HS) that contain viable hair matrix and outer root sheath keratinocytes as well as functional HFPU melanocytes are co-cultured in direct contact with inductive primary DP cells grown in 3D spheroids for 24 or 72 hours.

In this novel human folliculoid assay, DP cells retain their inductivity for 24-72 hours, as indicated by alkaline phosphatase activity and versican protein expression, and HS epithelium shows reduced number of apoptotic cells and higher proliferative activity in the residual hair matrix after co-culture with inductive primary DPs, as assessed by cleaved-caspase-3 or Ki-67 immunostaining, respectively, compared to plucked HS cultured without 3D DP spheroids. Moreover, in the presence of inductive HF mesenchyme, the residual melanocytes of the HFPU retain substantial melanogenic activity, as indicated by substantial tyrosinase activity in situ, melanin production, and gp100 expression. We are currently analysing how the absence or presence of HF mesenchyme impacts on the expression of hair keratins, IGF-1 and TGFβ2, and are probing the effects of selected hair growth- and/or pigmentation stimulatory agents.

This novel human folliculoid assay that combines heterologous DP spheroids and HSs from different donors in vitro offers a pragmatic and instructive medium throughput preclinical screening assay for candidate hair growth-/pigmentation promoting cosmeceuticals, nutraceuticals and drugs.

P216 | Dermal white adipose tissue undergoes major morphological changes during the spontaneous and induced murine hair follicle cycling

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Dermal white adipose tissue (DWAT) undergoes major fluctuations in thickness in association with the murine hair cycle. However, previous studies have provided conflicting hypotheses regarding the underlying mechanisms of DWAT changes. Therefore, we sought to elucidate whether the increased thickness in DWAT during anagen is mediated by hypertrophy (lipogenesis) or hyperplasia (genuine adipogenesis), and whether lipolysis or apoptosis can explain the decreased thickness of the DWAT during catagen. We also compared HC-associated DWAT changes in the spontaneous versus induced HC to distinguish between autonomous and hair follicle (HF) trauma-induced events. Here we find that fluctuations in HF size and downgrowth throughout the cycle do not explain the HC-dependent changes in DWAT thickness and area. Furthermore, we report that DWAT thickness is HC dependent but differs between spontaneous and depilation-induced telogen. Both dermal adipocyte (DA) proliferation and hypertrophy appear to be HC-dependent, whilst classical apoptosis within the DWAT appears absent throughout the HC—a phenomenon which currently remains unsolved. However, none of these fluctuations plausibly explain the HC-dependent changes in DWAT thickness. In contrast to previous studies, in vivo BODIPY uptake suggests that increased DWAT thickness during anagen occurs mainly via hypertrophy rather than hyperplasia, whereas DAs likely switch-on lipolysis as a mechanism for DWAT thinning during catagen. Hence, we propose that DWAT fluctuations in the spontaneous HC occur as a result of pre-adipocyte proliferation during mid-catagen to early anagen, followed by progressive, lipogenesis-driven DA hypertrophy during mid-to-late anagen, and lipolysis during catagen and telogen when DWAT thickness decreases. Interestingly, the induced HC exhibits increased DWAT thickness, area and DA number, but decreased DA volume/area compared to the spontaneous HC. Thus, DWAT shows additional, novel HF wounding-related responses during the induced HC, giving rise to a variety of research questions regarding the underlying molecular signals underpinning these responses. This systematic reappraisal provides important pointers for subsequent functional and mechanistic studies, and introduces the depilation-induced murine HC as a model for dissecting HF-DWAT interactions under conditions of wounding/stress. Lastly, our study raises the clinically-relevant question, whether and how recruiting wounding-induced HF signals in human skin may be exploited to therapeutically counteract conditions associated with lipoatrophy or undesired fat hyperplasia/hypertrophy by modulating DWAT morphology and function.

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P217 | Skin microbiota as potential trigger factors for pemphigus vulgaris

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Pemphigus vulgaris is a rare autoimmune blistering disease highly associated with certain HLA haplotypes and antibodies against desmoglein 1 and 3 leading to a disruption of the barrier function of the skin. Little is known about the factor(s) that trigger autoantibody production, and subsequently, lesions formation, in genetically susceptible individuals. Here, we aim to study differences in the skin microbiota of pemphigus vulgaris patients and first degree relatives. Ten families with at least three first degree relatives of German ancestry (n=47) were identified. From all patients and family members, blood was drawn for serology and DNA extraction and skin swabs were taken from five different body locations with different microenvironment. Nine of the clinically healthy first degree relatives had anti-Dsg3 IgG1 reactivity as determined by a modified ELISA (Euroimmun). Skin swabs were then processed for microbial DNA extraction and 16S rRNA gene sequencing (V1-V2 region) using the MiSeq Illumina platform. Taxonomic composition was found to be site specific between the selected sample locations. However, significant differences in diversity (alpha and beta) between patients and controls were only found for leg samples ($P<.05$). Potential family effects on the results could be ruled out using linear mixed effect modeling. Furthermore, three potential indicators showing significantly higher abundance in pemphigus vulgaris patients were identified (belonging to genera Staphylococcus, Dermabacter and Corynebacterium). We were able to show that the microbiome is influenced by pemphigus vulgaris. However, the effect of the disease on the microbiota is rather small and the results from this study should be verified using a larger cohort study.

P218 | Photosensitivity in patients with TREX1 associated lupus erythematosus as trigger for type I interferon induction and disease exacerbation

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Unrestricted accumulation of self-DNA in the cytosol is a danger signal stimulating the innate immune system. The DNA exonuclease TREX1 is located in the endoplasmatic reticlulum and acts on cytosolic DNA to prevent its accumulation. Mutations in TREX1 are associated with an increased risk to develop systemic lupus erythematosus or familial chilblain lupus. UV-induced oxidative DNA modification decreases the affinity of DNA for TREX1 restriction. Therefore, UV-irradiation could be a relevant trigger factor for disease flares in patients with reduced function of TREX1. To explore this hypothesis, we performed phototesting in patients with TREX1 associated SLE or familial chilblain lupus. All patients had a reduced photosensitivity for UVA and UVB. UV-irradiation enhanced ROS production and oxidative DNA damage in fibroblasts generated from patient skin. The DNA damage associated increase in cytosolic DNA led to an upregulation of type I interferon inducible genes upon UV-irradiation and rendered the cells more sensitive to type I interferon induction following additional stimulation with the viral mimic poly I:C. As type I interferons are potent immune stimulating cytokines favoring autoimmunity, the UV induced DNA damage could lead to disease flares in patients with mutation in TREX1 and explain their photosensitivity.

P219 | Skin microbiome analysis is affected by choice of DNA extraction kit

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Background: The skin microbiome has come into the focus of attention in the last years as alterations in its composition are observed in several skin diseases, e.g. atopic eczema, possibly hinting to its role in pathogenesis. However, there are several technical issues that make it problematic to correctly sequence the skin microbiome, usually by 16S rRNA sequencing. One problem is the relatively low load of bacteria on the skin, which consequently leads to low yields of bacterial DNA and potentially increases the risk of contaminations from other sources.

Aims: The aim of this study is to compare different DNA extraction kits from different manufacturers to assess their performance in DNA extraction and verify if there are kit-specific contaminations. This is important in order to establish a gold standard that will make skin microbiome analyses comparable between different studies and labs in the future.

Methods: A number of adjacent swabs were taken from the volar forearm of one individual and the bacterial DNA was isolated with different kits from different manufacturers. Furthermore, an artificial skin-analogue mock community was created, from which a dilution series with known numbers of cells were mixed together and their DNA was extracted with different extraction kits. Microbiome distribution was obtained by sequencing the variable regions V1-V3 of the 16S rRNA with the Illumina MiSeq platform.

Results: Beta diversity analysis (MDS and PCA) shows significant clustering as function of the DNA extraction kits used. Also, the alpha diversity of the skin microbiome is significantly different for different DNA extraction kits, even for swabs taken from adjacent sites of the volar forearm from the same individual. Furthermore, the sequencing of the mock community revealed differences in the performance of the different kits in extracting DNA from particular microbial families in a kit-dependent manner. Lastly, the results as function of the bacterial number dilution are under analysis and will be presented as well.

Conclusions: This study shows the importance of the choice of a good DNA extraction kit and adequate negative controls in order to obtain reliable microbiome data. Furthermore, different kits seem to preferentially extract some microbial families than others. Thus, the use of the same kit for all samples in the same study should allow reliable cross-sectional analysis, but even that might not ensure depicting the exact composition of the skin community.

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