43nd Annual Meeting of the Arbeitsgemeinschaft Dermatologische Forschung e.V (ADF)

Vienna, Austria, March 10–12, 2016

Local Organizers:

Clara Castellucci
Ahmad Jalili
Georg Stary
Georg Stingl

Program Committee:

Karin Loser
Kilian Eyerich
Ralf Ludwig
Martin Metz
Thomas Tueting
Amir Yazdi

For details see:
www.adf-online.de
For further information contact: info@adf-online.de

DOI: 10.1111/exd.12952
www.wileyonlinelibrary.com/journal/EXD

Allergy

P001

Dopamine agonists block mast cell degranulation

O. Schmetzer, P. Valentin and M. Maurer *Charité Universitätsmedizin Berlin, Department of Dermatology and Allergy, 10117 Berlin, Germany*

Mast cells (MC) are the key effector cells of allergic responses and responsible, at least in part, for the signs and symptoms of asthma, allergic rhinitis, atopic dermatitis and other allergic conditions. Following their activation by crosslinking of the high affinity receptor for IgE, FcRI, by IgE and antigen (allergen), MCs release multiple mediators, both preformed and newly synthesized. These cause vasodilation, sensory nerve activation and cellular influx by acting on endothelial cells, nerves, leukocytes and other cells.

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

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

P002

Serum levels of programmed cell death ligand-1 are increased in mastocytosis and correlate with disease severity

A. Rabenhorst[1], S. Leja[1], J. Schwaab[2], M. Gehring[3], A. Förster[1], A. Reiter[2], U. Raap[3] and K. Hartmann[1,4] *[1]University of Cologne, Department of Dermatology, Cologne, Germany; [2]Medical University of Mannheim, III. Medical Clinic, Hematology and Oncology, Mannheim, Germany; [3]Hannover Medical School, Department of Dermatology and Allergy, Hannover, Germany; [4]University of Luebeck, Department of Dermatology, Luebeck, Germany*

Mastocytosis is characterized by clonal expansion of mast cells (MC) associated with activating mutations of the KIT gene. Treatment options in mastocytosis are limited. Programmed death-1 (PD-1) is a key immune checkpoint receptor, which ensures Tcell tolerance by interacting with its ligands, PD-L1 and PD-L2. In a large variety of solid tumors and hematologic malignancies, tumor cells have been found to express PD-L1. Treatment of these tumors with antibodies against PD-1 or PD-L1 has been demonstrated to be highly effective.

In the present study, we aimed to investigate whether serum levels of PD-L1, PD-L2 and PD-1 are altered in patients with mastocytosis and whether these proteins are expressed in mastocytosis infiltrates.

Levels of PD-L1, PD-L2, PD-1, and tryptase were analyzed in serum of 43 patients with different categories of mastocytosis (adults, $n = 31$; children, $n = 12$) and 22 healthy controls (adults, $n = 10$; children, $n = 12$). PD-L1, PD-L2 and PD-1 levels were also measured in cell culture supernatant of the human mast cell line HMC1. Furthermore, bone marrow and skin biopsies were stained with antibodies against PD-L1, PD-L2, PD-1, and tryptase by immunofluorescence.

Serum levels of PD-L1 were significantly increased in adult patients with mastocytosis compared to adult healthy controls. Interestingly, patients with advanced disease categories exhibited significantly elevated PD-L1 levels compared to those with non-advanced categories. PD-L1 levels were also found to correlate with tryptase levels. Moreover, we detected significant levels of PD-L1 in supernatant of HMC1 cells and production of PD-L1 was more pronounced in HMC1.2 cells, which carry the mastocytosis-associated mutation KIT D816V, compared to HMC1.1, which lack this mutation. In contrast, we did not observe altered serum levels of PDL2 in adult patients, but a trend of increased PD-1 levels in patients with advanced mastocytosis categories. In bone marrow and skin biopsies of mastocytosis patients, expression of PD-L1 clearly colocalized with tryptase-positive MC. On the other hand, we did not observe colocalization of PD-L2-positive cells with tryptase-positive MC in both tissues. Whereas tryptase-positive MC in bone marrow failed to express PD-1, labeling in skin revealed marked expression of PD-1 on MC.

Together, our results demonstrate that adult patients with mastocytosis show increased serum levels of PD-L1, which correlate with severity of disease categories. These findings suggest investigating PD-L1 levels as diagnostic marker in patients with mastocytosis. Moreover, our data provide a rationale for exploring the efficacy of PD-1 and PD-L1 antibodies in the treatment of advanced mastocytosis.

P003

Updosing of non sedating antihistamines can improve the treatment of patients with cholinergic urticaria

K. Wosny[1], K. Weller[1], A. Werner[2], M. Maurer[1] and S. Altrichter[1] *[1]Charité – Universitätsmedizin Berlin, Department of Dermatology and Allergology, 10117 Berlin, Germany; [2]CharitéCrossOver CCO, Department for Physiology, Center of Space Medicine, Berlin, Germany*

Non sedating antihistamines (nsAH) are the first line treatment for cholinergic urticaria (CholU), a common form of inducible urticaria. In patients who remain symptomatic despite this treatment, the use of higher than standard nsAH doses is recommended. As of now, there is little published evidence to support this recommendation. Here, we assessed the efficacy of higher than standard dosed nsAH treatment in CholU in a real life setting. To this end, we measured disease activity (by symptom scores; Pruritus7 and UAS7), provocation threshold levels (by puls controlled ergometry), and quality of life impairment (by DLQI) in 30 CholU patients before and after seven days of treatment with increased doses (up to four fold) of nsAHs (Ceterizine, (Des-)Loratadine, Rupatadine, Ebastin, or Fexofenadine). Antihistamine updosing resulted in a significant reduction of diary based symptom scores (Pruritus7: −38%, $P = 0.002$; UAS7: −37%, $P = 0.003$). In contrast, quality of life improvement and the reduction of provocation thresholds were less pronounced and not statistically significant (DLQI: −21%, $P = 0.09$, PCE: −11%, $P = 0.08$). As assessed by responder analyses, 43% (UAS7) and 50% (Pruritus7) of CholU patients reported a reduction of their symptoms of more than 50%. Only one and two patients showed complete (90%) symptom control assessed by UAS7 and Pruritus7, respectively, in response to nsAH updosing. All investigated antihistamines were comparable in efficacy and safety. In summary, antihistamine updosing is effective in CholU patients, who are insufficiently treated with standard doses of nsAHs. Higher than standard nsAHs doses do not result in clinically meaningful or complete responses in all CholU patients subjected to nsAH updosing, and better treatment options need to be developed for these patients.

P004

In patients with cholinergic urticaria, atopy is common and linked to high disease activity and impact

S. Altrichter, K. Wosny and M. Maurer *Charité – Universitätsmedizin Berlin, Department of Dermatology and Allergology, 10117 Berlin, Germany*

Cholinergic urticaria (CholU) is a frequent and disabling disorder that presents with itchy wheal and flare-type skin reactions in response to physical exercise and passive warming. A higher frequency of atopy among CholU patients has previously been reported, but the significance of this observation is unclear. Here, we determined the prevalence of atopy in 30 CholU patients (Erlanger atopy score, EAS), and we compared atopic (aCholU) and non atopic (naCholU) patients for disease characteristics, activity (by urticaria activity score 7; UAS7) and severity (severity of disease score; OD), as well as quality of life impairment (by DLQI). More than half (57%) of the CholU patients analysed were found to be atopic as assessed by EAS (EAS categories 3 or 4). These aCholU patients, as compared to naCholU patients were mostly women (aCholU: 88%, naCholU: 43%, $P < 0.01$), more often reported frequent exacerbations in the summer (aCholU: 47%, naCholU: 31%, $P < 0.01$), and showed higher rates of sensitization to Candida albicans (aCholU: 24%, naCholU: 7%, $P = 0.036$). Both, disease activity and severity were higher in aCholU vs naCholU patients (median [range] UAS7: aCholU: 22 [4–42], naCholU 16 [4 −18], $P = 0.022$; median [range] SOD: aCholU: 15 [12–18], naCholU 14 [9 −17], $P = 0.046$). Also, quality of life impairment in aCholU patients was markedly increased as compared to naCholU patients (median [range] DLQI: aCholU: 11 [6–26], naCholU: 7 [1–15], $P = 0.046$). In summary, the prevalence of atopy is significantly increased in CholU patients and linked to higher disease activity, severity, and impact, i.e. quality of life impairment. Our results encourage the assessment of CholU patients for atopy and to further investigate and characterize the differences of CholU patients who are or are not atopic.

P005 (O05/02)

Exacerbation of allergen-induced gut inflammation in humanized mice by nutritional wheat alpha-amylase/trypsin inhibitors

I. Bellinghausen[1], B. Weigmann[2], V. Zevallos[3], S. Reissig[4], A. Waisman[4], D. Schuppan[3] and J. Saloga[1] *[1]University Medical Center of the Johannes Gutenberg-University Mainz, Department of Dermatology, 55131 Mainz, Germany; [2]University Hospital Erlangen, University of Erlangen-Nürnberg, Department of Internal Medicine I, 91052 Erlangen, Germany; [3]University Medical Center of the Johannes Gutenberg-University Mainz, Institute of Translational Immunology, 55131 Mainz, Germany; [4]University Medical Center of the Johannes Gutenberg-University Mainz, Institute for Molecular Medicine, 55131 Mainz, Germany*

The non-gluten proteins wheat alpha-amylase/trypsin inhibitors (ATIs), which have been identified as potent nutritional activators of various innate immune cells, are implicated as central triggers of wheat-induced asthma and gastrointestinal hypersensitivity to wheat. The aim of this study was to analyze whether ATIs are also involved in allergen-induced gut inflammation in a recently developed humanized mouse model of allergy. Therefore, nonobese diabetic-severe combined immunodeficiency-$\gamma c^{-/-}$ mice, receiving a gluten-free diet over at least three weeks before starting the experiment, were injected intraperitoneally with human PBMC from highly sensitized allergic donors together with the respective allergen or saline as control, and fed with different ATI-containing diets. After an additional allergen boost one week later, mice were challenged with the allergen rectally on day 21 and gut inflammation was monitored by a high-resolution video mini-endoscopic system evaluating translucency, granularity, fibrin production, vascularity, and stool. Allergenspecific human IgE in mouse sera, which was detectable only in PBMC plus allergentreated mice, was strongly enhanced in mice receiving an ATI-containing diet compared to mice which continued with the gluten-free diet. Consequently, allergeninduced IgE-dependent colitis was also enhanced in ATI-fed mice. Gut inflammation was even detectable in ATI-fed mice being injected with PBMC only in the absence of the respective allergen. These results underline that ATIs are important activators of food allergy which might be exploited for nutritional therapeutic strategies to address allergen- and gluten-induced intestinal and extraintestinal inflammation.

P006 (O01/02)

NOD2 signaling critically influences sensitization to orally ingested allergens and severity of anaphylaxis

T. Volz[1], F. Wölbing[1], F. Regler[2], S. Kaesler[1,2] and T. Biedermann[1] *[1]Department of Dermatology and Allergy, Technische Universität, Munich, Germany; [2]Department of Dermatology, University Hospital, Tuebingen, Germany*

Anaphylactic reactions to food are an increasing thread and are responsible for a rising number of emergency department visits and even related deaths. Food uptake occurs in a microbial rich environment in the intestine and alterations of organ specific microbiota have been shown to be associated with allergic and autoimmune diseases. Thus we sought to determine whether innate immune signals of the intestinal microbiota may influence sensitization to orally ingested allergens and subsequent anaphylactic reactions. Peptidoglycan (PGN) is a major cell wall component of both Gram-positive and Gram-negative bacteria which are abundantly found in the intestinal tract. PGN is sensed by the innate immune system by the pathogen-recognition receptors (PRR) NOD2 and TLR2, although the latter is still a matter of debate. To investigate the role of PGN recognition in regard to sensitization to food allergens, we sensitized C57Bl/6 and $TLR2^{-/-} \times NOD2^{-/-}$ mice with the model antigen Ovalbumin (OVA). After challenge with OVA, $TLR2^{-/-} \times NOD2^{-/-}$ mice showed significantly stronger decrease of core body temperature compared to wildtype mice, indicating that PGN recognition of the gut microbiota plays a dominant role in shaping susceptibility to food induced anaphylaxis. To delineate the impact of TLR2 and NOD2 in regard to PGN recognition, $TLR2^{-/-}$ or $NOD2^{-/-}$ mice were sensitized and challenged. Compared to wildtype mice $TLR2^{-/-}$ animals showed no differences in body temperature drop after challenge. In contrast $NOD2^{-/-}$ mice displayed a significantly stronger decrease in body temperature after challenge as wildtype mice resembling the results observed $TLR2^{-/-} \times NOD2^{-/-}$ double knock out animals. Furthermore $NOD2^{-/-}$ animals had significantly higher IgE levels than wildtype mice indicating that deficient recognition of PGN of the gastrointestinal microbiota by NOD2 leads to enhanced sensitization to orally ingested allergens and subsequently much more severe anaphylaxis after re-exposure. To examine the underlying mechanisms we isolated mesenterial lymph nodes from wildtype and knockout animals. After restimulation T cells from lymph nodes of $NOD2^{-/-}$ displayed significantly enhanced IL-4, IL-5 and IL-13 levels as measured by ELISA demonstrating predominant induction of T helper 2 cells in the gastrointestinal immune system (GALT) in the absence of NOD2 signaling. Interestingly splenic T cells and T cells from skin draining inguinal lymph nodes showed no difference between wildtype and $NOD2^{-/-}$ mice in regard to production of Th2 cytokines.

Taken together these results clearly demonstrate a critically role of innate immune recognition of PGN derived from the intestinal microbiota in shaping the quality of T helper cell responses in the intestinal immune system. NOD2 could be identified as the critical PRR sensing PGN. In its absence local but not systemic Th2 responses are induced in the GALT leading to enhanced IgE production and severe anaphylaxis. Activating NOD2 using either non-pathogenic bacteria or specific agonists could be a feasible strategy to attenuate sensitization to food allergens.

P007 (O01/01)

Soluble GARP inhibits allergic inflammation in humanized mouse model by enhancing Treg function

S. A. Hahn[1], H. Meyer-Martin[2], R. Buhl[2], H. Jonuleit[1], S. Reuter[2] and A. Tüttenberg[1] *[1]University Medical Center, Johannes Gutenberg University Mainz, Department of Dermatology, 55131 Mainz, Germany; [2]University Medical Center, Johannes Gutenberg University Mainz, III Medical Department, 55131 Mainz, Germany*

Regulatory T cells (Treg) play an essential role in maintaining immune homeostasis. Absence or impaired function of Treg can lead to autoimmunity and allergies. Glycoprotein A repetitions predominant (GARP) is an activation marker on the surface of human regulatory T cells (Treg). By modulating bioavailability of TGF-β, GARP is involved in the regulation of peripheral immune responses. We have recently shown that a soluble derivate (sGARP) has strong anti-inflammatory and regulatory properties *in vitro* as well as *in vivo*. Because modulations of Treg responses have therapeutic potential in inflammatory diseases, we investigated the impact of sGARP in treatment of allergic airway diseases using a humanized mouse model. In this model adult NOD/Scid gamma chain$^{-/-}$ mice received peripheral blood mononuclear cells (PBMC) from birch pollen allergic donors. To analyze the effects of sGARP, Treg alone or in combination with sGARP were transferred into the animals. After three weeks allergic airway disease was induced by a three-day intranasal challenge with birch pollen allergen. 48 h after last challenge allergic inflammation was assessed by measurement of airway hyperresponsiveness (AHR), quantification of cells in the bronchoalveolar lavage (BAL) and analysis of human immune cells in different tissues by flow cytometric and histological staining. The occurring inflammation could be blocked by Treg in a dose dependent manner. Additionally, in mice that received a non-protective ratio of Treg alone, additional transfer with sGARP significantly reduced AHR and immigration of inflammatory immune cells in the lung. sGARP had an effective anti-inflammatory impact also when administered repetitively. Furthermore, inhibition of allergic inflammation was abolished when mice additionally received TGF-β receptor II blocking agent, indicating that sGARP functionally depends on TGF-β signaling. In conclusion, our data show that sGARP significantly enhances the suppressive function of Treg and leads to an induction of Treg *in vivo*. Therefore, amplifying of Treg cell function via sGARP or repetitive application of sGARP alone seems to be a promising treatment option for allergic airway diseases.

P008

Pathogen recognition receptor-mediated immune processes in low zone tolerance to allergens

T. Schmidt[1], N. Lorenz[1], V. K. Raker[1], J. Roth[2] and K. Steinbrink[1] *[1]University Medical Centre Mainz, Department of Dermatology, 55131 Mainz, Germany; [2]University of Muenster, Institue of Immunology, 48149 Muenster, Germany*

Low zone tolerance (LZT) to contact allergens is concerned as a physiological mechanism for the regulation and circumvention of allergies. The tolerance reaction is induced by epicutaneous applications of subimmunogenic doses of haptens resulting in the generation of IL-10 producing $CD4^+CD25^+Foxp3^+$ Tregs and $CD8^+$ suppressor T cells, which in turn prevent the development of Tc1-mediated contact hypersensitivity (CHS), which mimics the human allergic contact dermatitis (ACD).

But the precise mechanisms of the innate immune system during the early phase of LZT, including activation of $CD4^+CD25^+Foxp3^+$ Tregs, are not yet understood. However, in CHS reactions, haptens have been shown to activate mechanisms of the innate immune system. In particular, hapten-stimulated TLR4 signalling is absolutely required for TNCB or nickel-induced CHS. Therefore, in this study, we have investigated the role of several TLR-related mechanisms in LZT. For this purpose, we used TLR4, TLR7, TLR9 and MRP14 KO mice, the latter one being deficient for the TLR4 ligands myeloid related proteins 8 and 14 (or known as alarmins S100A8 and S100A9). In order to induce a LZT, mice received several epicutaneous applications of subimmunogenic quantities of allergens (0.45 or 4.5 μg TNCB) onto the skin prior to sensitization and challenge with the same hapten. CHS mice served as controls, which developed a $CD8^+$ Tc1-mediated skin inflammation. Comparing TLR7 and TLR9 KO mice with control WT animals we did not observe any differences in the CHS reaction whereas TLR4 KO mice showed a reduced and MRP14 KO animals a significantly enhanced allergic cutaneous inflammation as previously described by other groups. However, in our study we found that LZT induction in the absence of TLR4, TLR7, TLR9 or MRP8/14, respectively, does not affect the development of the epicutaneously induced tolerance reaction and resulted in a significantly abolished CHS reaction, independent of the used subimmunogenic dose of the hapten. These results were determined by LZT-typical decrease of ear swelling (*in vivo*) after LZT induction in the KO mice, which was similar to that in WT controls. In addition, a diminished hapten-specific T cell-proliferation (*in vitro*) was observed in all used KO mice after LZT induction compared to CHS control mice which was comparable to WT mice. In addition, the experiments demonstrated a significantly reduced Th1-cytokine production (IFN-γ,IL-2) in mice lacking TLR4, TLR7, TLR9 or MRP8/14, respectively. Our data demonstrate that pattern recognition receptor-mediated processes in form of TLR4-, TLR9-, TLR7-, MRP8/14-induced mechanism of the innate immune system are not required for the induction of low zone tolerance to haptens and thus are not essential for prevention of CHS.

P009

D-Dimers are not a reliable biomarker for disease activity in chronic spontaneous urticaria patients

T. Ohanyan, S. Altrichter, N. Schoepke, M. Metz, M. Maurer and K. Weller *Charite – Universitätsmedizin Berlin, Department of Dermatology and Allergy, 10117 Berlin, Germany*

Background: The coagulation system has been repeatedly hypothesized to play a role in the pathophysiology of chronic spontaneous urticaria (CSU). D-dimer, a fibrin degradation product generated during fibrinolysis, is widely used in the diagnosis of thrombosis. Recently, D-dimer levels were reported to be increased during CSU exacerbations and to normalize in response to treatment.

Methods: In 73 patients with antihistamine-refractory CSU, D-dimer levels were determined and urticaria activity before (baseline) and after the initiation of treatment with omalizumab 300 mg (follow up) was assessed by the urticaria activity score of 7 consecutive days (UAS7). Response to treatment was globally classified by the treating physicians as complete response (>90% improvement), partial response (<90% and >30% improvement) and no response (<30% improvement).

Results: Mean D-dimer levels were found to be elevated before the initiation of omalizumab treatment. However, elevated D-dimer levels were present in only 53% of all patients and the variation of D-Dimer levels were high in subjects with comparable disease activity. Accordingly, the correlation of D-dimer levels with disease activity (UAS7) was found to be low ($r = 0.219$). After the first omalizumab injection mean D-dimer levels as well as mean UAS7 scores decreased. However, a decrease of D-dimer levels was present in only 62% of complete responders and 39% of partial responders, but also in 63% of non-responders. In addition, the correlation of D-dimer level changes from baseline to follow up with changes in urticaria activity were found to be low ($r = 0.147$).

Conclusion: Although mean D-Dimer levels are increased in CSU and decrease during improvement of symptoms, D-Dimers are not a suitable biomarker to reliably determine and monitor CSU disease activity in individual patients. Further studies are needed to determine if D-dimers are involved in the pathophysiology of CSU or if their elevation is an unspecific phenomenon downstream of mast cell activation.

P010

Role of protease-activated receptor 2- and tissue factor-mediated signaling in contact hypersensitivity

M. I. Schmidgen[1], T. Schmidt[1], N. Lorenz[1], S. Reyda[1], G. Carlino[1], W. Ruf[2] and K. Steinbrink[1] *[1]University Medical Center Mainz, Department of Dermatology, 55131 Mainz, Germany; [2]University Medical Center Mainz, Center for Thrombosis and Hemostasis (CTH), 55131 Mainz, Germany*

Protease-activated receptor 2 (PAR2) is activated by proteolytic cleavage of a broad array of extracellular proteases, including the binary complex of tissue factor (TF) and factor VIIa, and by indirect thrombin-induced PAR1 mediated cross-activation. Although the impact of these mechanisms on coagulation has been analyzed in detail, a deep understanding of their effect on the mechanisms of innate and adaptive immunity is still missing. However, several hints suggest that PAR2-/TF-mediated processes may be critically involved in cutaneous inflammatory disorders.

In the current study, we have thus investigated the role of PAR2 and TF in the murine model of the Tc1 CD8+ T cell-mediated contact hypersensitivity (CHS) which mimics human allergic contact dermatitis. For this purpose, we have generated a PAR2 receptor mutant mice, in which the substitution of arginine by glutamic acid results in a negative charge of the receptor cleavage site and, thereby, in insensitivity of PAR2 to proteolytic activation. However, the receptor is still present and thrombin-induced PAR2 activation via PAR1 cleavage does still occur. In a second approach, functional active TF was blocked *in vivo* by anti-TF antibody treatment (clone 21E10).

Our experiments revealed that the CHS reaction, induced by epicutaneous sensitization and challenge with the hapten 2,4,6-trinitrochlorobenzene (TNCB), was significantly diminished in both PAR2 receptor mutant mice and in anti-TF antibody treated mice compared to wildtype controls. These results were demonstrated by a reduced ear swelling *in vivo* and an impaired cellular infiltrate in the skin histologies of PAR2 receptor mutant mice and after TF blockade. In addition, we observed a reduced hapten-specific Tc1-mediated T cell response as shown by an impaired T cell proliferation and a decreased Tc1-cytokine production (IFN-γ, IL-2) after haptenspecific restimulation *in vitro* in PAR2 receptor mutant animals and in the absence of functionally active TF.

In summary, deficiency of PAR2 receptor signaling and inhibition of TF function results in an impaired T cell-mediated CHS reaction, indicating that PAR2-/TFmediated processes are required for immune responses in cutaneous allergic skin diseases.

P011

Detection of Bet v 1-specific IgG-producing cells in birch pollen-allergic patients and healthy controls by ELISPOT analysis

C. Baum, C. Möbs and W. Pfützner *Philipps University Marburg, Clinical & Experimental Allergology, Department of Dermatology and Allergology, 35043 Marburg, Deutschland*

Background: The role of allergen-specific IgG antibodies in IgE-mediated allergy is an important topic of recent research. While their induction during allergenspecific immunotherapy points to a potential function in mediating allergen tolerance, current studies show that allergen-specific IgG production is relatively common in both allergic individuals and healthy subjects, however, with different kinetics. To shed more light on IgG-dependent immune responses in immediate-type allergy, tools analyzing allergen-specific, IgG-secreting B cells would be very helpful. We here report on the development of a highly sensitive enzyme-linked immunospot (ELISPOT) assay for the detection of IgG antibody-secreting cells (ASC) in patients with birch pollen allergy and healthy controls.

Methods: Peripheral blood mononuclear cells (PBMC) isolated from either birch pollen-allergic patients or healthy control subjects were stimulated in different conditions to find the most efficient combination of reagents which induce total and allergen-specific IgG antibody production, the latter against Bet v 1 as the major birch pollen allergen. In addition, different cell numbers, incubation times and allergen concentrations were evaluated. To reduce background spot numbers two alternative detection systems were assessed, the conventional approach visualizing IgGASC by addition of biotinylated anti-IgG and a more sensitive procedure utilizing biotinylated Bet v 1 which directly binds to the secreted allergen-specific IgG of interest. Furthermore, allergen-specific IgG serum concentrations of patients and controls measured by ImmunoCAP were correlated with the number of IgG-ASC detected by the established ELISPOT assay.

Results: Combination of recombinant human (rh)IL-2 and toll-like receptor (TLR) agonist R848 proved to be the most suitable stimulus for the generation of Bet v 1- specific and total IgG-ASC whereas addition of rhIL-4 plus anti-CD40+/− CpG did not result in markedly elevated production of allergen-specific ASC. Comparison of birch pollen-allergic and healthy individuals showed that higher numbers of ASC were detected in the allergic patient cohort. In addition, preliminary data showed no correlation between the number of activated Bet v 1-specific B cells and the concentration of allergen-specific serum IgG addressing the need for assessment of both allergen-specific IgG levels and ASC frequencies, when analyzing IgG antibody responses in IgE-mediated allergy.

Conclusion: A highly sensitive ELISPOT assay for the detection of Bet v 1-specific IgG-ASC was established. Utilization of the assay allows the analysis of allergenspecific IgG responses on a single-cell level, thus providing further insights into the potential immunoregulatory role of B cells in patients with immediate-type allergy.

P012

Caterpillar dermatitis caused by setae of the oak processionary caterpillar: a clinical and histopathological study

H. Maier[1], T. Kinaciyan[1], C. Sinz[1], M. Grabenhofer[1], A. Schopf[2], I. Simonitsch-Klupp[3] and M. Susani[3] *[1]Medical University Vienna, Department of Dermatology, 1090 Vienna, Austria; [2]University of Natural Ressources and Life Sciences, Department of Entomology, 1190 Vienna, Austria; [3]Medical University Vienna, Clinical Institute of Pathology, 1090 Vienna, Austria*

The oak processionary caterpillar (OPC) is a forest pest, which feeds on certain oak species in Europe. Numerous 'poisonous' hairs (setae) protect 3. to 6. larval instar against predators, however, may become a thread to human health by direct contact or airborne spread. Setae (S) are hollow and contain the protein thaumetopoein. Investigation at present focusses on S as a cause for IgE-mediated reactions although caterpillar dermatitis presenting as contact dermatitis is the most frequent manifestation of OPC lepidopterism. We report clinical symptoms and histopathological features of a self-exposure experiment. This study is part of a scientific project funded by Deutsches Umweltbundesamt (UBA).

The author applied bundles of S harvested from living OPC to 3 distinct sites of the flexural side of the left forearm (L) by different means: manually, airborne, fragmented S by rotating pressure. He rubbed a fourth test area with the backside of a living OPC and applied S heated at 85°C for 30 min to 2 different skin areas (manually, airborne) on the right forearm (R). Our volunteer recorded clinical symptoms on both sides over 216 h. We took photos and laser scan microscopy images in 24 h intervals, punch biopsies at 48, 120, and 168 h follow-up, which we stained with H.E. and immunohistochemically methods. From the test areas on R we did not take punch biopsies.

After 3 h the skin reaction started on L with a diffuse flare-up followed by papules at 12 h. The rash changed its appearance from papules to vesicles at 48 h and began to spread to the surrounding skin. At 120 h the whole L including shoulder and lateral chest wall were affected. To our surprise, application of heated S caused a severe vesicular reaction (6 h) on R with heavy exudations, diffuse mild flare-up, but without spreading.

Acute dermatitis (48 h) shows massive intraepidermal vesicles or bullae arising from spongiosis and containing S, neutrophils, and monocytes in all epidermal layers. A dense inflammatory infiltrate consisting of eosinophils and CD68+ histiocytes typical for arthropod reaction (AR) is present in the upper dermis, and a pronounced perivascular lymphocytic infiltrate with eosinophils in its periphery extends into the deep dermis and partially into the subcutaneous fat. This infiltrate resembles Jessner-Kanof's lymphocytic infiltration of the skin (JK). Immunohistochemistry shows predominantly CD3+ T cells with a high amount of CD7+ NK cells. The CD4+:CD8+ ratio is 10:1, whereas only very limited B cells are present. In the subacute phase (120 h), the vesicles are smaller and contain material rich in protein and disintegrating neutrophils. Only few S are still detectable at that stage. Signs of both, AR and JK are still present. After elimination of S in the later stages (168 h), the epidermis shows pronounced reactive changes such as regenerative atypia and increased keratinocyt proliferation

at the site of healing vesicles. JK like infiltrate persists whereas histopathology loses the typical features of AR.
Clinical course and histopathological features of OPC dermatitis in L with superficial as well as deep lymphocytic infiltration and spongiosis make an underlying contact allergy to the content of S most likely. The reason for the irritant potency of heated S presenting clinically as irritant contact dermatitis, however, is not clear at present.

P013

Successful immunotherapy in a new mouse model of wasp venom allergy

C. Hosp[1], N. Beyersdorf[2], S. Kranz[2], A. Trautmann[1], J. Stoevesandt[1], C. Adam[1], M. Goebeler[1] and A. Kerstan[1] *[1]University Hospital Wuerzburg, Department of Dermatology, Venereology and Allergology, 97080 Wuerzburg, Germany; [2]University of Wuerzburg, Institute for Virology and Immunobiology, 97078 Wuerzburg, Germany*

Background: In human wasp venom allergy specific immunotherapy (SIT) is the only causal therapy of this IgE-mediated allergic disease reaching an efficacy of up to 95%. It is believed that allergen tolerance during the early phase of SIT is largely mediated by IL-10-producing regulatory T cells (Treg), which balance Th1 (IFN-γ) and Th2 (IL-4, IL-5) immune responses. However, the in-depth investigation of cellular and molecular mechanisms of SIT in humans is hampered by ethical and methodology constrains.
Objective: We studied clinical features as well as *in vivo* and *in vitro* immune responses in wasp venom allergic mice before and during SIT.
Materials and methods: BALB/c mice were first sensitized to wasp venom followed by SIT on three consecutive days. The efficacy of SIT was investigated by a standardized wasp venom challenge; the outcome was evaluated by an anaphylaxis scoring system. Mouse mast cell protease-1 (MCPT-1), a serum marker for IgEmediated mast cell degranulation, was monitored. Furthermore, *in vitro* IgEsensitization to wasp venom was tested by basophil activation test (BAT) and T cell responses were investigated by wasp venom-specific proliferation assays as well as cytokine measurements from culture supernatants.
Results: Wasp venom injection in sensitized mice led to anaphylaxis accompanied by an increase in serum MCPT-1. Wasp venom-specific IgE-sensitization of mice was demonstrated *in vitro* by positive BAT and wasp venom-specific IL-4 and IL-5 secretion in T cell cultures. In analogy to humans, SIT protected mice efficiently from anaphylaxis. During SIT we found attenuated wasp venom-driven T cell proliferation, a significant increase in IL-10 production, and a diminished allergen-specific IL-5 secretion of T cells.
Conclusions: In our newly established mouse model of wasp venom allergy SIT protects mice from IgE-mediated anaphylaxis. Not only the clinical response, but also the immune response patterns induced by wasp venom SIT were comparable to the human situation opening a path for in-depth investigation of unknown issues in SITinduced immune tolerance.

P014

Reduction and hyperreleasability of lytic T Cell granules in atopic asthma – correlation with lung function parameters

A. Pasckert[1,2], B. Bonnekoh[1], H. Gollnick[1], N. Waldburg[2], J. Schreiber[2] and A. Ambach[1] *[1]Otto-von-Guericke-University Magdeburg, Clinic for Dermatology and Venereology, 39120 Magdeburg, Germany; [2]Otto-von-Guericke-University, Clinic for Internal Medicine, Department of Pneumology, 39120 Magdeburg, Germany*

The perforin-containing lytic granule system of cytotoxic lymphocytes was shown to be severely altered in patients with exacerbated atopic dermatitis (AD) or with allergic rhinoconjunctivitis (RCA) in the off pollen season. Namely, a significant reduction of perforin-containing cytotoxic T lymphocytes (CTL) and a reduced perforin load of these cells were demonstrated. In addition, following activation, perforin-granules were released significantly faster and more complete in AD as compared to healthy controls (HC), a phenomenon called peforin-hyperreleasabilty.
Conflicting results, however, are reported in the peripheral blood of patients with atopic asthma (AA), namely, an augmentation as well as a reduction of perforincontaining lymphocytes. Functional data regarding releasability of lytic granules are lacking.
Therefore, the lytic granule system was characterized in 15 patients with nonexacerbated AA and in 15 HC individuals. Patients with systemic immuno suppression were excluded. The lytic granule system of peripheral lymphocytes was analyzed on a single cell basis using monoclonal antibodies against perforin and granzyme B as marker molecules. In addition, release velocity of lytic granules as induced by cell activation with ionomycin and PMA was determined by immuno-flow cytometry. Data were obtained using a FACS-Scan applying the cellquest software (Becton Dickinson, Heidelberg). SPSS.22 was used for statistical analysis.
Our data demonstrate: (i) In AA-patients, significantly fewer peripheral lymphocytes contained perforin as compared to HC confirming and extending previous results. (ii) Perforin$^+$ CTLs outnumbered granzyme B$^+$ CTLs in AA and HC. Both granule-types differed in their release kinetics: perforin comes first which makes sense biologically. This phenomenon is not reported previously. (iii) For the first time, hyperreleasability of lytic granules in AA is shown, i.e. CD8$^+$ CTLs of patients released both, perforin- and/or granzyme B-containing granules approximately twice as fast than CTL from HC. Significant correlations with lung function parameters were detected (Pearson, bivariant, correlation coefficient $r \geq \pm 0.5$, $P \leq 0.05$): Perforin$^+$ portion of CD8$^+$ CTLs 30 min and 60 min after activation – FEV1%. Absolute number of perforin$^+$ CD8$^+$ CTLs – FVC % and FEV1/FVC. Perforin$^+$ portion of CD56$^+$ lymphocytes – VC, FVC, FEV1/VC, FEV1/FVC and FEV1/IVC.
One may conclude: 1) Granule-reduction and -hyperreleasability is a pan-atopic phenomenon. 2) Since the lytic granule system is known to be involved directly in IgE-control, alterations described here may contribute to IgE-deregulation in AA. 3) Correlative evidence suggests that the lytic granule system plays a role in AA-lung pathology. This is supported by a recent report of an asthma mouse model where allergen-specific CTL required perforin expression to suppress allergic airway inflammation.

Cellular Biology

P015

Insights in the substrate-specifity of ADAM17, the main EGFR-ligand sheddase

M. Munz, K. Koyro, M. Sperrhacke, A. Sommer and K. Reiss *University of Kiel, Department of Dermatology, 24105 Kiel, Germany*

A disintegrin and metalloproteinase 17 (ADAM17) is the most important sheddase of epidermal growth factor receptor (EGFR)-ligands. The protease releases, inter alia, tumor growth-factor-alpha and amphiregulin. Therefore, this enzyme is a key regulator of epithelial homeostasis, migration and proliferation. Accordingly, lack of ADAM17 in mice results in embryonic lethality accompanied by severe skin defects. Recently, first human patients were described suffering from a loss-of-function mutation in the ADAM17 gene leading to resembling epithelial defects. Dysregulation of ADAM17 is linked to diverse cutaneous diseases and disturbed epidermal barrier function. The aim of this work was to deepen the understanding of the shedding event, in particular with regard to the domain structure of ADAM17. One aspect of the ADAM-dependent substrate cleavage is that both, enzyme and substrate, need to be in close proximity. Analyzing the structure of ADAM17 and its substrates, a potential role of their transmembrane-domains can be postulated. To find out, whether there is a potential protein-interaction site within the transmembrane region, we used mutagenesis studies and analyzed the release of different EGFR-ligands. Indeed, we identified a potential interaction motif for some substrates. However, our data indicate that there is not one common motif for all substrates. Instead, we propose that the ADAM17 transmembrane region contributes to the recognition of specific substrates such as amphiregulin, while other domains might be responsible for the binding of other EGFR-ligands. Increased knowledge about the substrate selectivity of ADAM17 could lead to deeper insights into epithelial diseases caused by dysregulation of this important protease.

P016

A combination of in-silico and in-vitro models helps understand the dynamics of the Senescence Associated Secretory Phenotype

P. C. Meyer[1], P. Maity[1], C. Müssel[2], K. Singh[1], F. Ferreira[1], L. Krug[1], H. J. Maier[3], M. Wlaschek[1], T. Wirth[3], H. A. Kestler[2] and K. Scharffetter-Kochanek[1] *[1]Ulm University, Department of Dermatology and Allergic Diseases, 89081 Ulm, Germany; [2]Ulm University, Medical Systems Biology, Ulm; [3]Ulm University, Institute of Physiological Chemistry, Ulm*

Cells are subjected to continual stresses from exogenous and endogenous sources. These events can cause a number of responses, ranging from complete recovery to malfunction and ultimately cell death. Permanent cell-cycle arrest or senescence is a protection mechanism that helps cells recover from this damage and seems to be a fundamental mechanism of aging, wound healing and development. However, cellular senescence can be accompanied by a senescence associated secretory phenotype (SASP) that causes chronic inflammation and paracrine senescence. While senescence in general is proposed to be beneficial for wound healing, the SASP is not and can be cause for temporary or chronic wound healing disorders. There are indications that senescence is causal for chronic venous leg ulcers, explaining why the severity and occurrence is higher in aged individuals. We additionally propose that it is not only the amount of preexisting senescent cells but also the developing SASP that determines the onset of a chronic wound and the outcome of wound healing.
Here we present a core gene regulatory network of the development and maintenance of senescence and the SASP incorporating published gene expression and interaction data of different signaling pathways like IL-1, IL-6, p53 and NF-κB under the assumption of DNA damage or oncogenic stress.
The modeled simulations correspond to published data on cellular senescence and the SASP. Furthermore we can predict different in-silico knock-outs that prevent key SASP-players, like IL-1, IL-6 and IL-8, from getting activated upon cell cycle arrest. In a first screening we found different gene knock-outs and knock-out combinations that prevent the activation of IL-6 signaling, a factors that among others seems to be responsible for spreading and retaining the SASP. In this way we could single out the NF-κB Essential Modifier (NEMO) as a potential target. Under the assumption of DNA damage, a NEMO-knockout was enough to prevent the activation of IL-6 and IL-8 in-silico.
To validate these in-silico results in-vitro we used a NEMO-floxed mouse to isolate murine dermal fibroblasts that were afterwards transfected with a Cre-expressing plasmid including a fluorescence-reporter-construct to enable FACS-sorting of positively transfected cells. In these cells we introduced Etoposide-mediated DNA damage and subsequently analyzed mRNA expression and protein secretion of IL-6 and murine IL-8 homologues CXCL1 (KC, GRO-1), CXCL2 (MIP-2) and CXCL5 (LIX, GCP-2) using qPCR and ELISA. Verifying our in-silico results, we could show that a NEMO knockout inhibits IL-6 and all three IL-8 homologue mRNA expression and protein secretion in murine dermal fibroblasts after DNA damage in-vitro, possibly enabling us to at least lower the contagiousness of the SASP for neighboring cells.
Consequently the combination of in-silico models and in-vitro benchwork gives us the power to create in-vitro and in-vivo models that might help to understand the dynamics of the SASP and other processes and can be used to broaden our understanding of highly important wound healing mechanisms.

P017

Antimicrobial effects of EDA- and TAEA-functionalized celluloses in three dimensional skin models infected with Candida albicans

S. Finger[1], S. Fink[1], D. Winter[1], C. Wiegand[1], T. Liebert[2], T. Heinze[2] and U. Hipler[1] *[1]Universitätsklinikum Jena, Jena; [2]Friedrich-Schiller-Universität Jena, Jena*

Introduction: Rendering celluloses antimicrobially active can be achieved by functionalization through introduction of positively charged groups. For investigation of these new potential antimicrobials, 3d skin models consisting of a dermis and epidermis serve as suitable models after infection with Candida albicans to determine effects under *in vivo* like conditions. It is hypothesised that celluloses (FC) are able to protect 3d skin models from yeast invasion and that the antimicrobial activity depends on the degree of substitution (DS) and functional group.
Methods: Celluloses were functionalized with ethylendiamine (EDA) or triaminotriethylamine (TAEA) and differ in the DS (0.35–0.56). 3d skin models were infected with C. albicans DSM 1386 or with C. albicans ATCC MYA-2876. One hour later they were incubated with different FC. PBS and 0.5% SDS served as controls. Supernatants were collected after 24 h or 48 h after incubation with C. albicans and FC for quantitative measurement of IL-1α, IL-6 and IL-8, determination of cytotoxic effects by LDH measurement and analysis for yeast growth. Expression rates of IL-1α, IL-1β, IL-6, IL-8, IL-18, TNF-alpha, hBD2, hBD 3 and LL-37 were examined with qPCR. The skin models were further subjected to histological analyses.
Results: EDA-FC showed higher cell compatibility than TAEA-FC. Independent from functional group, FC concentrations tested were not adequate to complete inhibit yeast growth under the current test conditions.
Conclusions: Recently, it could be shown that the antimicrobial efficacy of FC against C. albicans depends on the degree of substitution and the functional group as does the biocompatibility. In accordance, EDA-FC showed higher cell compatibility than TAEA-FC. However, the FC concentrations here tested were not effective to kill yeast cells in the 3d skin models, which are more complex and may influence the activity of FC caused by the interplay with different cell types and specific cell interactions, under the current test conditions. Further studies will elucidate if the FC will prohibit C. albicans infections of the 3d skin models when applied prior to yeast exposure *in vitro*.

P018

Biocompatibility of Sap from leaves of Isatis tinctoria and several active compounds in a co-culture model of human HaCaT keratinocytes and Arthroderma benhamiae

J. Hesse[1], C. Wiegand[1], M. Grün[2], N. Fankhaenel[2], R. Schleicher[3], M. Keiner[3] and U. Hipler[1] *[1]Universitätsklinikum Jena, Jena; [2]Food GmbH Analytik – Consulting, Jena; [3]Nuth GmbH & Co.KG, Mihla*

Introduction: Woad, Isatis tinctoria L., is known for its blue indigo dye and for its antimicrobial and anti-inflammatory properties. Active compounds, such as tryptanthrin, are thought to exhibit a strong antibacterial activity as well as possess antimycotic properties. The present study analyses the bioactivity and biocompatibility of woad sap and several active compounds using an *in vitro* coculture model of human HaCaT keratinocytes and Arthroderma benhamiae (Ab.).
Methods: Antimycotic activity of two saps of fresh leaves (filtered (F); non-filtered (NF)) and several compounds e.g. tryptanthrin (T); indican (I); indole-3-carbinol (I3C) and ferulic acid (FA) against A.

benhamiae was determined by microplatelaser- nephelometry (BMG Labtech). Determination of the cellular ATP content (ATPLite(TM)-M, PerkinElmer) provided information on the HaCaT viability. Bioactivity was analysed using an *in vitro* co-culture model of HaCaT and Ab. Quantification of Ab. was carried out by measuring the fluorescent intensity after staining the fungal cell walls with calcofluor white.
Results: Half maximal lethal (LC50) and inhibitory concentrations (IC50) were determined in regard to cell compatibility and antimicrobial activity. The sap of fresh leaves F (LC50 = 9.1%) showed higher cell compatibility than NF (LC50 = 7.1%). The antifungal activity against Ab. was slightly higher for F (IC50F = 2.3%; IC50NF = 2.1%). LC50 of the methanol extracts I, I3C, and FA (LC50 ≥ 60 μg/ml) and of the DMSO extract T (LC50 = 6 μg/ml) are considerably higher than their IC50 against Ab. The ratio of LC50/IC50 for all test materials is >1, reflecting a high antimycotic activity at concentrations harmless to the cells. Higher concentrations of all test materials were necessary to inhibit Ab. in the co-culture model. Active compounds exhibited a strong cell protective effect with T > I > I3C > FA.
Conclusions: All test materials exhibited good biocompatibility against HaCaT keratinocytes with a high antifungal activity against A. benhamiae. These results are crucial evidence that woad could be a natural source for antimycotic agents with a high biocompatibility.

P019

Influence of extracellular matrix molecules on the release of adiponectin in adipose-derived stem cells

S. Schreiner, L. Petry, M. Butting, K. Steinhorst, N. Zöller, M. Hofmann, R. Kaufmann, M. Meissner and S. Kippenberger *Hospital of the J.W. Goethe University, Dept. of Dermatology, Venereology and Allergology, 60590 Frankfurt, Germany*

Subcutaneous fat contains stem cells (adipose-derived stem cells, ADSC) which can be transdifferentiated *in vitro* into a variety of cell species including osteocytes, chondrocytes, myocytes, endothelial cells, epithelial cells or adipocytes. In the present study we isolated ADSCs from abdominal subcutaneous fat and investigated the impact of extracellular matrix molecules (ECM) on the release of adiponectin, a prominent adipokine. At first, the obtained cell population was characterized by stemness-associated antigen markers (CD31−, CD34+, CD45−, CD54−, CD90+, CD105+, CD166+, HLA−ABC+, HLA−DR−) using FACS. Then, ADSCs were transdifferentiated into adipocytes by specific medium supplements on different ECM (collagen I, laminin, hyaluronic acid). In relation to regularly used polystyrol supports our data show that collagen I and laminin decrease the expression of adiponectin as detected by ELISA whereas hyaluronic acid had no effect. These preliminary findings indicate that ECM molecules are modulators of adiponectin expression and therefore may contribute to metabolic regulation.

P020

Epithelial transdifferentiation of adipose-derived stem cells (ADSC) – Comparison of different medium compositions and the effect of ECM proteins on transdifferentiation

L. Petry, S. Schreiner, M. Butting, K. Steinhorst, N. Zöller, M. Hofmann, A. Bernd, R. Kaufmann, M. Meissner and S. Kippenberger *Hospital of the J.W. Goethe University, Department of Dermatology, Venereology and Allergology, 60590 Frankfurt, Germany*

Background: Adipose-derived stem cells (ADSC) hold great promise for regenerative medicine, as they are easily isolated and possess a multilineage differentiation potential. In the ongoing study, different culture conditions were compared and optimized, prompting the transdifferentiation of ADSCs into epithelial-like cells.
Methods: ADSCs were isolated from abdominal subcutaneous fat tissue and characterized by flow cytometry. Then, ADSCs were cultured for 7 days in different media triggering transdifferentiation. Finally, the expression of pan-cytokeratin as a first indicator for epithelial differentiation was measured by flow cytometry. For further characterization also other epithelial markers (keratin 5/14, involucrin, E-cadherin and desmoglein) will be examined by immunofluorescence, Western blot analysis, flow cytometry and qPCR.
Results: From a variety of different media supplements tested, the combination of all-trans retinoic acid, bone morphogenetic protein-4, hydrocortisone, fetal bovine serum, epidermal growth factor and L-ascorbic acid 2-phosphate triggered the most successful transdifferentiation as measured by pan-cytokeratin expression. Ongoing experiments test the presence of other differentiation markers and the impact of extracellular matrix proteins such as collagen IV, laminin, hyaluronic acid and fibronectin.
Discussion: Optimized conditions for the transdifferentiation of ADSCs into epithelial cells might be helpful in the treatment of non-healing wounds by promoting the reepithelialization process.

P021

Non-keratinocyte SNAP29 influences epidermal differentiation and hair follicle formation in mice

S. Schiller[1], C. Seebode[1,2], G. Wieser[3], S. Goebbels[3], T. Ruhwedel[3], M. Horowitz[4], D. Rapaport[4], O. Sarig[5], E. Sprecher[5] and S. Emmert[1,2] *[1]University Medical Center Goettingen, Department of Dermatology, Venereology and Allergology, 37077 Göttingen, Germany; [2]University Medical Center Rostock, Clinic for Dermatology and Venereology, 18057 Rostock, Germany; [3]Max-Planck-Institute for Experimental Medicine, Department of Neurogenetics, 37077 Göttingen, Germany; [4]Tel Aviv University, Department of Cell Research and Immunology, Tel Aviv, Israel; [5]Tel Aviv Sourasky Medical Center, Department of Dermatology, Tel Aviv, Israel*

The human CEDNIK (CErebral Dysgenesis, Neuropathy, Ichthyosis, Keratoderma) syndrome, a rare neurocutanous disorder, is caused by loss-of-function mutations in the SNAP29 gene. The corresponding SNAP29 is a SNARE (SNAP (Soluble NSF Attachment Protein) REceptor) protein that mediates intracellular membrane fusion processes and turned out to be necessary for epidermal differentiation. We recently reported the successful generation and characterization of total ($Snap29^{-/-}$) and keratinocyte specific (Snap29 fl/fl/K14-Cre) Snap29 knockout mice. In this current study we extended our investigations and revealed subtle differences in epidermal differentiation and hair follicle morphogenesis between both mutant mouse lines. In contrast to wild type and epidermal Snap29 knockout mice, exclusively the stratum corneum of $Snap29^{-/-}$ mice showed parakeratosis. In electron micrographs we detected higher numbers of electron lucent vesicle-like structures. These structures presumably represent non-secreted, malformed lamellar bodies. Both mutant lines showed organelle remnants in lower stratum corneum cells but in $Snap29^{-/-}$ epidermis the amount of these remnants was increased compared to Snap29 fl/fl/K14- Cre stratum corneum. Furthermore, an evaluation of histological samples showed a stronger reduction of hair follicles in $Snap29^{-/-}$ mice. In $Snap29^{-/-}$ skin we found a 50% reduction versus wild type skin compared to a 19% reduction versus wild type in Snap29 fl/fl/K14-Cre mice skin. Together, these findings are indicative for more pronounced disturbances in the epidermal differentiation of $Snap29^{-/-}$ skin and a contribution of non-keratinocyte SNAP29 to the composition and organization of the epidermis and epidermal appendages, like hair follicles. We assume that disturbances in primary cilia formation that interfere with mesenchymal-epidermal crosstalk can be responsible for the observed subtle phenotypic differences and especially the reduced number of hair follicles in $Snap29^{-/-}$ epidermis.

P022

Comparative transcriptomic analysis in rosacea subtypes display features of the same disease complex without consecutive evolution

T. Buhl[1,2], M. Sulk[3,2], F. Cevikbas[2], V. Schwab[3], P. Novak[3], J. Buddenkotte[3], C. Kempkes[2], J. Aubert[4], J. Voegel[4] and M. Steinhoff[5,2] *[1]UMG Dermatology, Göttingen, Germany; [2]UCSF Dermatology, San Francisco, USA; [3]UKM Dermatology, Muenster, Germany; [4]Galderma R&D, Sophia Antipolis, France; [5]UCD Charles Institute, Dublin, Ireland*

Rosacea is a common, chronic inflammatory skin disease of poorly understood origin. Based on its clinical features (flushing, burning, chronic inflammation, fibrosis) and trigger factors, a complex pathobiology involving dysregulation in the immune, vascular, and nervous system can be anticipated.
To identify the distinct and commonly dysregulated genes in the different rosacea subtypes, we analyzed whole-transcriptome expression profiles in patients with erythematotelangiectatic rosacea (ETR), papulopustular rosacea (PPR), phymatous rosacea (PhR) and compared with healthy volunteers. In ETR, dysregulated lipid metabolism and activation of the innate immune system represent the most dysfunctional gene groups, whereas PPR patients display predominantly a complex activation of multiple pathways of innate and adaptive immunity. PhR patients revealed many similarities with PPR gene analyses, but additional genes involved in tissue remodeling in association to inflammation were significantly elevated. In contrast, epidermal growth factor and Wnt signaling family members show diminished expression profiles.
Strikingly, comparison of ETR, PPR, and PhR with healthy volunteers reveals dysregulations in the same identical gene sets in 49–72% of all altered genes, depending on rosacea subtype. Thus, the three rosacea subtypes are closely related manifestations of the same disease complex; however, conclusions drawn from our data do not support a distinct, linear evolution of the disease (e.g., beginning with ETR, development to PPR, and finally occurrence of PhR). This study is the most comprehensive analysis of rosacea pathophysiology to date and highlights several new candidates for possible therapeutic interventions.

P023 (O06/04)

Tight Junctions are impaired in diabetic keratinocytes – implications for barrier function and wound healing

C. Ueck, T. Volksdorf, S. Vidal-y-Sy and J. M. Brandner *University Hospital Hamburg-Eppendorf, Department of Dermatology and Venerology, Hamburg, Germarny*

Diabetes mellitus type II is a common metabolic disease which is often associated with impaired wound healing and increased susceptibility to infections. Tight Junctions (TJ) are important for skin barrier function and TJ proteins are altered during skin infection and wound healing. Therefore, we wanted to know whether TJs/TJ proteins are altered in diabetic keratinocytes. Indeed, we observed a downregulation of the TJ proteins Claudin-1, Claudin-4 and Occludin in nonlesional skin from patients with diabetes type 2 compared to age, sex and body location matched healthy controls on mRNA levels and/or in immunofluorescence intensity. There was no clear alteration for ZO-1. Also in cultured keratinocytes derived from patients with diabetes type 2 the downregulation of Cldn-1 and Cldn-4 was preserved. Looking for TJ/skin barrier functionality, we observed a significant decrease of transepithelial resistance, i.e. barrier function to ions, as well as increased permeability for 4 and 40 kDa tracer molecules in cultured keratinocytes from diabetic donors. Supplementation of healthy primary keratinocytes with high glucose concentrations did also impair TJ barrier function. There was no clear effect when blocking the insulin-receptor to mimic insulin resistance. Concerning wound healing, we observed that downregulation of Cldn-1 and Occludin resulted in delayed scratch wound healing in normal keratinocytes under normal (Cldn-1) or stressed (Occludin) conditions.
In conclusion, we found impaired TJ barrier function in diabetic keratinocytes which is likely to contribute to increased susceptibility to skin infections. Further we could show that Cldn-1 and Ocln play an important role in cutaneous wound healing and their downregulation in diabetic skin is likely to contribute to impaired wound healing in patients with diabetes.

P024

Cutis laxa acquisita – novel biochemical insights into defective elastogenesis

P. Gkogkolou[1], K. Hildebrandt[2], S. Broekaert[1], D. Metze[1], G. Sengle[2] and M. Boehm[1] *[1]Department of Dermatology, 48149 Muenster, Germany; [2]Center for Biochemistry, Medical Faculty, and Center for Molecular Medicine Cologne (CMMC), University of Cologne, 50931 Cologne, Germany*

Cutis laxa is a very rare disorder of defective elastic fiber formation. In contrast to inherited forms of this disease where mutations in distinct genes have been unraveled the pathogenesis of cutis laxa acquisita (CLA) remains poorly understood. We report on a 37-year-old woman with progressive CLA and paraproteinemia. Systemic involvement was not found. One year before skin manifestation the patient was diagnosed with membranoproliferative glomerulonephritis and had received oral corticosteroids in combination with torasemide, a loop diuretic, and candesartan, an angiotensin-II-receptor antagonist. Family history was unremarkable. Histology from skin biopsy specimens confimed the diagnosis of cutis laxa. To shed light into the pathogenesis of this case we established fibroblast cultures from affected and normal-appearing skin of the patient and analyzed them for protein expression and immunolocalization of key components of elastogenesis. Immunofluorescence analyses showed a normal assembly of fibrillin-1 and latent transforming growth factor-β (TGF-β) binding protein-1 (LTBP-1) fiber networks in both strains. Although there was no difference in the intracellular levels of these proteins, their secretion in the extracellular space was reduced by 60% in affected cells, as shown by Western blot analysis. Intracellular production of fibulin-5 and LTBP-4 was normal, but their secretion was reduced by 70% for fibulin-5 while completely inhibited for LTBP-4. Moreover, glycosylation of LTBP-4 was impaired. Assembly of extracellular LTBP-4 or fibulin-5 networks could not be detected in both cell strains *in vitro*, which could be due to the age of the patient. Interestingly, in affected cells the Golgi apparatus (GA) appeared to be abnormally shaped and contained deposits of intracellular accumulated fibulin-5 and LTBP-4. The proliferation rate of these cells was markedly decreased. Based on these findings we hypothesize that inhibition of LTBP-4 secretion due to a GA dysfunction has led to impaired elastogenesis in our patient. The reason for this GA alteration remains unclear but medication-induced GA dysfunction could be a possible explanation. Interestingly, it has been previously shown that that genetic ablation of LTBP-4 leads to severe defects in elastogenesis. Our work describes for the first time – to the best of our knowledge – a GA dysfunction as the probable cause for CLA and further points to LTBP-4 as an important player for maintaining postnatal elastic fiber formation.

P025

Barrier formation and wound healing capacity of human keratinocytes are strongly influenced by culture medium and predetermined by donor source

M. Zorn-Kruppa[1], T. Volksdorf[1], C. Ueck[1], E. Zöller[1], P. Houdek[1], K. Reinshagen[2], I. Ridderbusch[2], G. Bruning[3], I. Moll[1] and J. M. Brandner[1] *[1]University Medical Center Hamburg-Eppendorf, Department of Dermatology and Venerology, 20246 Hamburg, Germany; [2]University Medical Center Hamburg-Eppendorf, Department and Clinic of Paediatric Surgery, 20246 Hamburg, Germany; [3]Tabea Hospital, 22587 Hamburg, Germany*

The use of primary human keratinocytes has been reported for different applications ranging from monolayer analysis to fabrication of complex 3D skin equivalents for different scientific or clinical purposes. To replace fetal bovine serum, various keratinocyte-specific serum-free culture media have been developed and are nowadays distributed by commercial cell culture companies. However, only few comparative studies exist on the influence of different serum-free culture media on keratinocytes and more particularly on parameters like barrier properties. Therefore, we explored the influence of three frequently-used culture media (KGM-2, DermaLife, and EpiLife) on keratinocyte barrier function by quantifying the transepithelial resistance, the permeabilities for two tracers of different size as well as tight junction protein localization and expression. In addition, scratch wound closure rates under normal and high-glucose conditions were compared in keratinocytes cultured in the different media. Furthermore, we assessed the impact of inter-individual variability for selected parameters by analysing different keratinocyte donors. In summary, our studies demonstrate a great impact of the medium as well as strong donor derived inter-individual variability regarding barrier formation and wound healing capacity of keratinocytes. This clearly shows that medium as well as donor-derived differences have to be kept in mind when analyzing the degree of barrier function, and comparison between studies as well as transfer to clinical situations is only possible when these differences are taken into account.

P026

Preventing the cutaneous inflammatory response using a bacterial endopeptidase

M. C. Stock, C. Hillgruber, B. Pöppelmann and T. Goerge *University Hospital of Muenster, Department of Dermatology, 48149 Muenster, Germany*

A major event in leukocyte recruitment to the site of inflammation is the binding of endothelial selectins to their sialylated glycoprotein-ligands on the leukocyte to mediate rolling and prime the leukocyte for integrin trans-activation. The Osialoglycoprotein endopeptidase (OSGEP) produced by the bovine lung pathogen Mannheimia Haemolytica specifically cleaves O-sialoglycoproteins. Here, we investigate putative anti-inflammatory properties of OSGEP *in vitro* and *in vivo*.

Using two *in vivo* models of cutaneous inflammation, we observed significant reduced edema formation and leukocyte infiltration at the sites of inflammation after intravenous OSGEP application, indicating its efficiency in suppressing the cutaneous inflammatory response. OSGEP-treated bone marrow-derived neutrophils (BMN) did neither show altered vitality, receptor-mediated activation nor chemotactic capacity. However, we found decreased BMN transendothelial migration that was not due to altered endothelial permeability. Intravital microscopic assessment of leukocyte-endothelial interactions prior to extravasation revealed reduced rolling and adhesion of OSGEP-pretreated BMN in the vasculature, as well as diminished recruitment into the inflamed skin. Comparable reduction of BMN rolling and adhesion was found under shear flow on endothelial cells *in vitro*. Interestingly, OSGEP pretreatment of endothelial cells did not show additive reduction of leukocyte-endothelial interaction, indicating OSGEP to act rather on leukocytes than on endothelial cells. Moreover, OSGEP-treated BMN tend to roll faster and over longer distances compared to vehicle-treated and show reduced integrin-mediated adhesion to ligand-coated surfaces. These results suggest that OSGEP impairs major steps of extravasation.

Our findings demonstrate that OSGEP reduces cutaneous inflammation, most likely by reduction of rolling and adhesion on activated endothelium, and therefore decreasing the capability of leukocytes to extravasate. OSGEP could bear great potential as a therapeutic agent in cutaneous inflammatory diseases.

P027

ADAMTS13, a specific von Willebrand factor (VWF)-cleaving protease, regulates VWF-mediated increase of cutaneous vascular permeability

C. Hillgruber[1], B. Pöppelmann[1], D. Vestweber[2], S. W. Schneider[3] and T. Goerge[1] *[1]University Hospital of Muenster, Department of Dermatology, 48149 Muenster, Germany; [2]Max Planck Institute of Molecular Biomedicine, Vascular Cell Biology, 48149 Muenster, Germany; [3]University Hospital of Mannheim, Department of Dermatology, 68167 Mannheim, Germany*

Von Willebrand factor (VWF), a well-known prothrombotic factor, is increasingly recognized as a pro-inflammatory protein. Previously, we demonstrated that VWF is an important regulator of both neutrophil-mediated (immune complex-mediated vasculitis (ICV) and irritative contact dermatitis (ICD)) and T-cell-mediated (DNFBinduced contact hypersensitivity (CHS)) cutaneous inflammation.

Here, we studied the role of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type I motif-13) for VWF-mediated cutaneous inflammation. In the circulation, VWF multimers are strictly controlled by ADAMTS13 which constitutively and specifically cleaves ultralarge VWF strings into smaller, less adhesive multimers. Recent studies have shown that ADAMTS13 has protective effects against ischemic brain damage and reduces VWF-mediated acute cerebral inflammation following stroke. However, an involvement of ADAMTS13 in cutaneous VWF-mediated inflammation is yet unknown.

First experiments reveal a role of ADAMTS13 for vascular permeability in the skin. Vascular leakage which was either induced by application of a vasodilator (histamine or bradykinin) or by neutrophil-mediated inflammation (ICV and ICD) was significantly increased in ADAMTS13$^{-/-}$ mice compared to WT control mice. In addition, VWF plasma levels were significantly higher in inflamed ADAMTS13$^{-/-}$ mice compared to inflamed WT mice. However, ADAMTS13 deficiency did neither enhance vascular leakage in T-cell-mediated inflammation (CHS) nor impair the overall clinical response in all studied models of cutaneous inflammation. Analysis of the neutrophil-specific enzyme myeloperoxidase and histology revealed that there was no difference in neutrophil or T-cell infiltration into inflamed skin of ADAMTS13$^{-/-}$ mice compared to WT control animals.

Ongoing research will investigate the putative regulatory role of ADAMTS13 for VWFmediated cutaneous vascular integrity in more detail.

In conclusion, targeting VWF provides a therapeutic anti-inflammatory approach for treatment of diverse cutaneous inflammatory diseases and might be – at least to strengthen vascular integrity – implemented by therapeutic substitution of the VWFcleaving protease ADAMTS13.

P028

Mesenchymal stem cells regulate T cell functions in chronic venous leg ulcers

D. Jiang[1], J. Muschhammer[1], S. Schatz[1], P. C. Meyer[1], A. Bánvölgyi[2], K. Lörincz[2] and K. Scharffetter-Kochanek[1] *[1]University of Ulm, Department of Dermatology and Allergic Diseases, 89231 Ulm, Germany; [2]Semmelweis University, Department of Dermatology, Venereology and Dermatooncology, 1085 Budapest, Hungary*

Persistent inflammation is the prime cause of chronic wounds such as chronic venous leg ulcers (CVU), which severely affect the quality of life of patients with an increase in overall morbidity and mortality and is associated with a heavy social-economic burden. So far, the persistent inflammation in CVU are commonly recognized to be caused by overactivated macrophages and neutrophils. Emerging studies have suggested that T cells such as epidermis-resident memory effector T cells, dermis-resident memory regulatory T cells and $\gamma\delta$ T cells may also play important roles in inflammatory chronic wounds. Mesenchymal stem cells (MSCs) have shown promising anti-inflammatory properties including suppressing effector T cell functions and induction of regulatory T cells. In this study, using a full-thickness excisional wound model with iron-overload mice that mimic important pathogenic aspects of human CVU, we found that iron-overload wounds had more T cells at basal level compared to wild type wounds. Intradermal injection of MSCs reduced the numbers of CD4+ and CD8+ T cells compared to PBS injected wounds. *In vitro*, the proliferation of regulatory T cells grown in the presence of soluble anti-CD3 antibody and recombinant IL-2 was significantly enhanced by cocultured MSCs. In addition, the polarization of CD4+ T cells was influenced by cocultured MSCs. The populations of regulatory T cells (CD4 + CD25 + Foxp3+) were substantially expanded with the presence of MSCs, while the populations of Th1 (CD4 + IFN-γ+) and Th2 (CD4 + IL-4+) were slightly but significantly reduced. *in vivo*, using the allogenic dendritic cell-expanded MSC-instructed regulatory T cells were found to be home to iron-overload wounds after adoptive transfer by i.v. injection. The future work will focus on elucidating the identity and function of T cell subpopulations in human CVU and the murine iron-overload wound model, and exploring the effect of MSCs on these T cell subpopulations for potential treatment options for difficult-to-heal CVU.

P029 (O02/02)

TNF dependent apoptosis is the major but not the sole mechanism involved in the development of skin disease in cFLIP deficient mice

D. Panayotova-Dimitrova[1,2], M. Feoktistova[1,2], M. Ploesser[2] and M. Leverkus[1,2] *[1]Medical Faculty Aachen, RWTH Aachen, Department of Dermatology, Aachen, Germany; [2]Section of Molecular Dermatology, Department of Dermatology, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany*

The outcome of death receptor triggering is decided by the stoichiometry and activation of a number of critical signaling proteins such as FADD, caspase-8, and cellular FLICE-inhibitory protein (cFLIP). These molecules are core proteins in the respective membrane-bound or intracellular cell death signaling complexes, evidenced by the importance of their presence during embryonic development. Organ-specific deletion of either FADD or caspase-8 results in a pronounced inflammatory skin disease presumably caused by increased necroptosis or other receptor-interacting protein (RIP)-kinase 3 (RIPK3)-dependent signals. We previously showed that inducible epidermis-specific ablation of cFLIP leads to embryonic lethality. When cFLIP was abrogated postnatally, acute loss of cFLIP led to massively increased apoptosis of keratinocytes and furthermore to the development of an inflammatory skin disease that requires the presence of TNF. As a response to loss of cFLIP, TNF mRNA expression and protein secretion were highly upregulated, arguing that a TNF-mediated autocrine loop in the skin is the cause of TNF-mediated apoptosis of cFLIP-deficient PK *in vivo*. To give further credential to our *in vivo* data, we have now generated TNF$^{-/-}$/cFLIPfl/fl/K14CreERTam animals. Intriguingly, ablation of TNF rescued the phenotype of cFLIP deficiency from characteristic weight loss and increased mortality observed in TNF+/+/cFLIPfl/fl/K14CreERTam mice. Moreover the lack of TNF in these animals strongly reduced and delayed epidermal hyperkeratosis and increased apoptotic cell death as determined by histological analysis of cleaved caspase-3. Cytokine analysis of separated epidermis and dermis demonstrated that upregulation of inflammatory cytokines such as IL-1β or IL-8 was repressed in the absence of TNF. Additionally we have now generated RIPK3$^{-/-}$/cFLIPfl/fl/K14-CreERtam animals which similarly to the TNF$^{-/-}$/cFLIPfl/fl/ K14CreERTam animals lacked the weight loss and subsequent mortality. However, cell death induction in the skin was unaltered as determined by active caspase-3 staining. In addition cytokine analysis of the epidermis showed unaltered induction of IL-1β or IL-8 cytokines in these animals, arguing for a RIPK3-independent upregulation of inflammatory cytokines upon deletion of cFLIP.

Taken together our data suggest that TNF-dependent apoptosis is a major mechanism of epidermal cell death whenever cFLIP is unable to protect TNFmediated death. However, alternative pathways involving RIPK3-dependent cell death signaling may also contribute to the development of the dramatic skin disease and lethality upon cFLIP deletion. Our findings provide evidence for a negative regulatory role of cFLIP for epidermal inflammation and subsequent TNF-dependent apoptosis in the skin. Our data warrant future studies of the regulatory mechanism controlling the development of skin disease upon cFLIP deficiency and the role of cFLIP in a number of skin diseases including toxic epidermal necrolysis (TEN).

P030

Biodentine®, a dentine substitute, reduces collagen type I synthesis on RNA and protein level in pulpa fibroblasts

N. Zöller[1], F. Nikfarjam[1,2], M. Butting[1], D. Heidemann[2], R. Kaufmann[1], A. Bernd[1] and S. Kippenberger[1] *[1]University Hospital, Department of Dermatology, Venereology and Allergology, 60590 Frankfurt, Germany; [2]University Hospital, Department of Restorative Dentistry, 60590 Frankfurt, Germany*

Investigations concerning the influence of externally added compounds to cells of the oral cavity are relevant for dentistry as well as dermatology as many dermatologic diseases also affect the oral mucosa. The development of biocompatible and bioactive materials in dental medicine desires the preservation of patient's own teeth with the necessity to characterize the material properties.

Aim of this study was to investigate the effects of Biodentine® on primary pulp cells focusing on collagen synthesis on protein- and RNA level.

Biodentine® was solved according to the manufacturer's instructions; the emerging paste was spread on a silicon molding tool to obtain discs with a diameter of 5.1 mm. Biodentine® discs were incubated in culture medium without cells and the media were collected and replaced every day for 5 days. Primary pulp cells isolated from freshly extracted wisdom teeth of 20–23 years old patients were treated with these eluates for

8–24 h. In supernatants we analysed the protein concentration of the N-terminal domain of pro-collagen type I (P1NP) and TGF-β. Additionally we investigated the influence of Biodentine® on the gene expression of collagen type I analysing the RNA concentrations of two specific collagen type 1 genes (Col1A1 and Col1A2).

We found a maximum downregulation of P1NP release, which is a measure of collagen type I synthesis, by 93% for cultures treated with three or five discs. Similarly in qPCR the expression of genes of the two respective collagen type I chains (Col1A1, Col1A2) were decreased by 45% after 16 h and was no more detectable after 24 h. A Biodentine® dependent decrease of TGF-β secretion could be observed for the cultures treated with the eluates of three or five Biodentine® discs. Serial dilution of the five Biodentine® discs containing eluates substantiated these observations.

In summary in our studies Biodentine® eluates reduced the TGF-β secretion as well as collagen type I synthesis. The relevance for the clinical application has to be further investigated in more complex systems.

P031

The transcriptional repressor Trim28 is a key factor for the establishment of a functional epidermis

L. Beer[1,2], T. Berger[1], E. Tschachler[1] and M. Mildner[1] *[1]Medical University of Vienna, Dermatology, 1090 Vienna, Austria; [2]Medical University of Vienna, Biomedical Imaging and Image-guided Therapy, 1090 Vienna, Austria*

The development of a functional epidermis is a complex process involving precise adjustment of gene expression controlled by the activation of transcriptional regulators.

To identify factors that might be involved in terminal keratinocyte (KC)-differentiation we performed gene chip analysis of differentiated human KC in monolayer cultures and compared them to KC differentiated in skin equivalent models (SE). Our bioinformatics analysis revealed that 272 mRNAs

were differentially expressed between these two KC-differentiation models. These genes, presumably important for late epidermal development, were mainly involved in the biological processes of extracellular matrix formation, collagen fibril organisation, cell adhesion and epidermal development. Based on these transcriptomic data, we were able to identify the transcriptional repressor Trim28 as a putative key factor regulating the expression of genes selectively modulated in SE. Trim28 is known to promote histone methylation (H3K9me3) leading to selective repression of gene expression. To verify these data, we performed siRNA mediated knock-down of Trim28 in primary KC and established SE with these cells. Indeed, Trim28 deficient SE showed an impaired development of the epidermis, displaying a significantly reduced thickness of the living layers. Gene chip analysis of the Trim28 knock-down SE revealed a strong upregulation of 34 genes which were mainly associated with proteolysis and extracellular matrix disassembly, confirming the involvement of Trim28 in epidermal development. In addition, we could demonstrate that Trim28 contributes to immunological processes and epidermal defence mechanisms by regulating the expression of specific antimicrobial peptides, including beta-defensins and RNAse7.
Together, this study identified Trim28 as a key factor for the establishment of a functional epidermis. Our transcriptomic data provide a basis for the identification of additional, so far unknown molecules important for epidermal homeostasis.

P032

Antimicrobial peptides target mTOR signaling in keratinocytes in psoriasis

C. Buerger[1], V. Lang[1], S. Diehl[1], E. Hattinger[2], R. Kaufmann[1] and R. Wolf[2] *[1]University Hospital of the Goethe University, Frankfurt; [2]Ludwig-Maximilians-Universität, Munich*

The mTOR (mechanistic target of rapamycin) pathway is a central regulator of cell growth and differentiation, which is hyperactivated in acanthotic psoriatic skin. In psoriasis, the mTOR kinase itself is strongly activated in basal epidermal layers, while a downstream target of mTOR, the ribosomal protein S6, is hyperactivated in suprabasal differentiating layers of psoriatic skin lesions. However, disease-intrinsic factors which regulate epidermal mTOR activity are mostly unknown. Koebnerisin (S100A15) is an innate anti-microbial and immune-modulatory peptide strongly upregulated in the psoriatic epidermis. Here, data revealed that the localization of koebnerisin resembles the activation pattern of the mTOR kinase. We hypothesized a functional link and unveiled that koebnerisin was capable of activating Akt and mTOR signaling in normal human keratinocytes. Further, koebnerisin conferred a small effect on keratinocyte proliferation, which emphasizes previous results on the minor role of mTOR signaling in regulating epidermal proliferation.
In addition, we showed that aberrant activation of mTOR by Th1/Th17-cytkines or S100 peptides leads to reduced expression of differentiation markers, such as keratin1, involucrin or filaggrin. Conversely, regular differentiation can be restored under these conditions if mTOR signalling is blocked through siRNA mediated knockdown. Together, our data link the innate immune factor koebnerisin and Akt/ mTOR signaling with epidermal maturation in psoriasis and suggest potential targets to control chronic inflammatory diseases in the skin and beyond.

P033

K14-Cre-mediated deletion of Atg7 leads to accumulation of sequestosome 1 in Merkel cells

S. Sukseree, H. Rossiter, E. Tschachler and L. Eckhart *Medical University of Vienna, Research Division of Biology and Pathobiology of the Skin, Department of Dermatology, 1090 Vienna, Austria*

Merkel cells and epidermal keratinocytes develop from common keratin K14- expressing precursors. Here, we used the Atg7f/f K14-Cre mouse model to delete Atg7, a regulator of the lysosomal degradation pathway known as autophagy, and to compare the effects of the abrogation of autophagy in both cell types. Transgenic GFP-LC3 labeled autophagosomes in keratinocytes and Merkel cells of wildtype mice but not in the equivalent cells of Atg7f/f K14-Cre mice. Immunofluorescence labeling showed that the deletion of Atg7 leads to the accumulation of sequestosome 1/p62 in Merkel cells but not in keratinocytes of the whiskers and the plantar skin. Sequestosome 1 is a key regulator of several cellular processes, including the capture of autophagy substrates in autophagosomes. Interestingly, we detected high levels of sequestosome 1 also specifically in Merkel cells within human skin biopsies, suggesting that the Atg7f/f K14-Cre mouse model could mimic some aspects of sequestosome-1-dependent processes in human skin. Taken together, these results suggest that autophagy contributes to the homeostasis of Merkel cells *in vivo*.

P034 (O04/06)

Proteomic identification of autophagy substrates in cornifying keratinocytes

K. Jaeger[1], V. Mlitz[1], S. Zhong[1], S. Sukseree[1], H. Rossiter[1], R. Rice[2], E. Tschachler[1] and L. Eckhart[1] *[1]Medical University of Vienna, Research Division of Biology and Pathobiology of the Skin, Department of Dermatology, Vienna, Austria; [2]University of California Davis, Department of Environmental Toxicology, Davis, CA, USA*

Cornification of keratinocytes is a special form of programmed cell death during which structural proteins are cross-linked and organelles are broken down. We hypothesized that the cellular self-digestion program known as autophagy might contribute to the degradative processes in cornification. To test this hypothesis, the essential autophagy gene Atg7 was deleted by the Cre-lox system specifically in keratin K14-expressing cells, such as keratinocytes of the epidermis and the nail apparatus. Cornified nails were isolated, digested and subjected to proteomic analysis. The abundance of proteins was compared between nails from fully autophagy-competent mice and mice lacking autophagy in keratinocytes. The suppression of autophagy led to the significant accumulation of diverse types of enzymes, proteasomes, chaperonins, and proteins involved in cell motility. By contrast, the amounts of cytoskeletal proteins of the keratin family, keratin-associated proteins and desmosomal proteins were either unaltered or slightly decreased in Atg7-deficient versus normal nails. Changes in protein abundance were not caused by alterations in the levels of gene expression. Taken together, the results of this study demonstrate that autophagy degrades a broad spectrum of noncytoskeletal proteins in cornifying keratinocytes and thereby shapes the proteome of nail corneocytes.

P035

Histamine down-regulates psoriasin (S100A7) and calprotectin (S100A8/S100A9) in a human skin equivalent model

M. Gschwandtner, A. Tschachler, B. Lindner and E. Tschachler *Medical University of Vienna, Research Division of Biology and Pathobiology of the Skin, 1090 Vienna, Austria*

Keratinocytes contribute to the barrier function of the skin by expression of (I) epidermal differentiation proteins, e.g. filaggrin and loricin, (II) tight junction proteins like occludin and claudin and (III) antimicrobial peptides such as beta-defensins and S100-proteins. We have shown previously that the mast cell mediator histamine suppresses the expression of (I) epidermal differentiation proteins and (II) tight junction proteins in keratinocytes. In the present study we investigated the hypothesis that histamine also influences the expression of (III) antimicrobial peptides.
Keratinocytes were cultured under different growth conditions: proliferating monolayer cultures, differentiated (postconfluent) monolayer cultures and in three dimensional skin equivalent models. Experiments were performed in presence or absence of histamine and/or selective histamine receptor agonists and antagonists. The mRNA expression of the antimicrobial peptides HBD1, HBD2, HBD3, S100A7, S100A8, S100A9, RNase5, RNase7 and LL-37 was investigated by real-time PCR and protein expression was analyzed immunohistochemically.
The expression of antimicrobial peptides was elevated in differentiated monolayer cultures as compared to proliferating monolayer cultures and an additional strong increase was observed in skin equivalent models. We confirmed previous findings that histamine up-regulates the expression of HBD2 and HBD3 in proliferating monolayer cultured keratinocytes. In differentiated monolayer cultures histamine did not influence the expression of any of the investigated antimicrobial peptides. In the three dimensional skin equivalent model histamine down-regulated the expression of S100A7, S100A8 and S100A9 at the mRNA and protein level. A selective histamine H1 receptor agonist reduced the expression of S100-proteins similar to histamine and pre-incubation with a histamine H1 receptor antagonist abolished the effect of histamine. Agonists and antagonists specifically binding the other histamine receptors (H2R, H3R and H4R) were ineffective.
Our findings demonstrate that in addition to reducing the expression of late differentiation and tight junction proteins histamine also down-regulates the expression of a subset of antimicrobial peptides; histamine thereby modulates skin barrier function in diverse ways.

P036 (O03/04)

Disturbed protein homeostasis in Cockayne syndrome – a circulus vitiosus may cause premature aging

M. C. Alupei, K. Scharffetter-Kochanek and S. Iben *University of Ulm, Dermatology and Allergic Diseases, 89081 Ulm, Germany*

Cockayne syndrome (CS) is a progeria characterized by childhood onset of degenerative symptoms reminiscent of the aging body as loss of subcutaneous fat, alopecia, cataracts, neurological degeneration and cachexia, accompanied with developmental delay resulting in a severe phenotype ('cachectic dwarfs') witch can lead to childhood death. It is a model disease of 'accelerated' aging and its exploration should foster our understanding of the 'normal' aging process. CS can be caused by the recessive mutation of 5–6 genes that are all involved in a branch of Nucleotide-Excision Repair (NER), thus explaining the elevated UV-sensitivity of the patients, however, total loss of NER is not necessarily followed by premature aging suggesting that a loss of alternative functions of the CS-proteins may cause premature aging. One common alternative function of at least 5 CS-proteins is transcription of the ribosomal RNA by RNA polymerase I. Here we show that a disturbed RNA polymerase I transcription is followed by a decreased translational fidelity at the ribosomes and oxidised proteins initiating endoplasmic stress that elicits a unfolded protein response that in turn represses RNA polymerase I transcription. Oxidative hypersensitivity- a hallmark of CS cells and the pathophysiologic difference to cells with the mild UV-sensitive syndrome, which can also be caused by mutations in some CS proteins, can be overcome by chemical chaperones. Moreover, chemical chaperones can break the circulus vitiosus and restore RNA polymerase I transcription and growth of CS-cells. As these chaperones are approved by the FDA for the treatment of neurodegenerative diseases, our findings imply a possible treatment for a devastating childhood disorder and may have impact to our understanding of the aging process itself.

P037

Map kinase p38alpha stress signaling repairs skin barrier defects caused by loss of insulin/IGF-1 signaling

E. Wachsmuth[1,2], S. Y. Aghdam[1,3], C. Günschmann[4,1], M. D. Akyüz[1,2], M. Pasparakis[5,2], J. C. Brüning[6,4] and C. M. Niessen[1,2] *[1]University of Cologne, Department of Dermatology, 50931 Cologne; [2]University of Cologne, Cologne Excellence Cluster on Cellular Stress Responses in Aging-associated Diseases (CECAD), 50931 Cologne, Germany; [3]University of Cologne, Center for Molecular Medicine Cologne, 50931 Cologne, Germany; [4]University of Cologne, Center for Endocrinology, Diabetes and Preventive Medicine, 50931 Cologne, Germany; [5]University of Cologne, Institute for Genetics, Cologne, Germany; [6]Max Planck Institute for Metabolism Research, 50931 Cologne, Germany*

The epidermis of the skin provides the organism with a crucial physical, chemical and immune barrier, which in mice is formed between embryo day (E) 16.5 and birth. Recently we showed that epidermal Insulin receptor (IR) and Insulin-Like Growth Factor receptor (IGF-1R) signaling (IIS) controls p63 dependent stratification early during epidermal morphogenesis. To examine whether IIS directly control epidermal barrier function we combined trans epidermal water loss (TEWL) and toluidine blue penetration assays. Loss of epidermal IIS resulted in a strongly impaired barrier function early during development, which improved significantly around birth. At E16.5 filaggrin expression and processing and active caspase 14 levels were strongly impaired, indicating that IIS directly controls the formation of a functional stratum corneum. However, both fillagrin and caspase processing were restored to almost control levels late in epidermal barrier development, suggesting the activation of a compensatory pathway upon loss of IIS. Interestingly, epidermal loss of IIS resulted in the activation of p38 MAPK stress kinase already at E16.5. We next asked whether this activation was functionally relevant. Mice with combined epidermal loss of p38α and IGF-1R showed a much more severe barrier dysfunction as loss of IGF-1R alone as measured by e.g increased.TEWL and TLSP expression. As loss of p38α alone did not cause an obvious skin barrier defect, these data indicate that activation of p38α loss repairs the stratum corneum skin barrier defects induced by loss of IIS. In agreement, newborn p38α/IGF-1Repi$^{-/-}$ mice still showed a defect in filaggrin processing in contrast to IGF-1R or p38α single knockout mice. To identify mechanisms by which p38α promotes barrier formation upon loss of IIS we performed unbiased SILAC spike in proteomics on newborn epidermis and found that many proteins encoded by the epidermal differentiation complex that control proper formation of the stratum corneum were strongly altered upon loss of p38α and IGF-1R. In conclusion our data identify a key role for IIS in the regulation of the stratum corneum skin barrier and this skin barrier dysfunction may contribute to skin related pathology in type II diabetes patients. Moreover, our analysis revealed a novel and unexpected role for p38map kinase stress signaling in rescuing a developmental skin barrier defect. At present we are examining whether this pathway functions as a general mechanism to compensate for skin barrier dysfunction.

P038 (O03/03)

Classical cadherins control skin barrier function through polarized actin organization and junctional EGFR receptor localization and activation

M. Rübsam[1], A. Mertz[2], A. Kubo[3], B. Boggetti[1], E. Dufresne[2], V. Horsley[2], M. Amagai[3] and C. M. Niessen[1] *[1]University of Cologne, Dermatology, 50931 Cologne, Germany; [2]Yale University, 06520 New Haven, CT, USA; [3]Keio University School of Medicine, Dermatology, 160-8582 Tokyo, Japan*

Classical cadherins are key determinants of intercellular junction formation and apico-basolateral polarization in simple epithelia. In the epidermis junctions are polarized across the different layers, with barrier forming tight junctions (TJs) in the most upper viable granular layer (SG). How polarization is controlled in the epidermis and whether classical cadherins are involved is not known. Previously, we showed that epidermal E-cadherin is essential for epidermal TJ barrier function. To examine epidermal junctional organization across the layers in 3D we combined staining of epidermal whole mounts and high resolution microscopy. This revealed that whereas E-cadherin is in adherens junctions across all layers, vinculin, recently implicated in mechanotransduction across cadherins, was

only recruited to adherens junctions in the stratum granulosum (SG). This coincided with a strong polarized organization of F-actin across epidermal layers with only strong cortical organization of F-actin observed in the SG. More importantly, the SG2 layer itself is highly polarized despite its flattened appearance with a lateral AJ network reaching up to a continuous ZO-1 positive apical tight junctional ring. Interestingly, staining for the EGFR receptor revealed a strong enrichment near tight junctions at the SG2. This polarized organization of vinculin, F-actin and EGFR was lost in E-cadherin$^{-/-}$ epidermis, accompanied by discontinuous ZO-1 staining and increased EGFR activation. Interestingly, *in vitro* E-cadherin$^{-/-}$ primary keratinocytes showed impaired recruitment of vinculin and ZO-1 to early adhesion zippers resulting in decreased intercellular adhesion force and tight junctional dysfunction. This coincided with increased internalization of the TJ component occludin and the EGFR accompanied by ERK map kinase activation. Importantly, inhibition of EGFR rescued TJ barrier function and EGFR internalization. At present we are testing whether inhibition of EGFR rescues *in vivo* skin barrier function and perinatal lethality upon loss of E-cadherin. Together, our data indicate that E-cadherin controls the polarized organization of junctions, cytoskeleton and receptor signaling in a mechanosensitive manner to coordinate epidermal barrier formation and function. Our data also identify a novel role for junctional EGFR signaling in controlling skin barrier homeostasis, which may have important implications for a range of barrier related diseases.

P039

Thy-1/β3 integrin interaction-induced apoptosis of dermal fibroblasts is mediated by up-regulation of FasL expression

M. Schmidt[1], D. Gutknecht[1], K. Anastassiadis[2], B. Eckes[3], U. Anderegg[1] and A. Saalbach[1] [1]*University of Leipzig, Department of Dermatology, Venereology and Allergology, 04129 Leipzig;* [2]*Dresden University of Technology, Biotechnology Center, Dresden;* [3]*University of Cologne, Department of Dermatology, Cologne*

The control of the balance between cell proliferation and apoptosis of fibroblasts is crucial for maintaining tissue homeostasis, physiological wound healing/ scar formation and prevention of tissue fibrosis or tumour progression. Recently, we reported that the glycoprotein Thy-1 contributes to the maintenance of skin homeostasis by suppressing proliferation and promoting apoptosis of dermal fibroblasts via interaction with β3 integrins. In the present study we investigated the mechanisms of Thy-1/ β3 integrin mediated control of programmed cell death in fibroblasts. Interestingly, skin fibroblasts from Thy-1 deficient mice showing less apoptosis displayed decreased FasL expression. Next, blocking of Thy-1/ β3 integrin interaction on wildtype (wt) fibroblasts resulted in down-regulation of FasL expression to the level of FasL in Thy-1$^{-/-}$ fibroblasts. Blocking of Fas/ FasL induced apoptosis in wt fibroblasts completely reversed Thy-1 mediated effects on cell proliferation and apoptosis whereas no effects were observed in Thy-1$^{-/-}$ fibroblasts. Our data indicate that the interaction of Thy-1 with β3 integrin stimulates FasL expression resulting in enhanced apoptosis and reduced cell growth of dermal fibroblasts.

To underline the data, Thy-1 dependent FasL expression was investigated *in vivo*. Previous analysis of healing full thickness skin wounds in Thy-1$^{-/-}$ mice and wt controls displayed more proliferating and less apoptotic cells in granulation tissue of Thy-1$^{-/-}$ mice 7 days post wounding. In this study we established a fibroblastspecific knockout mice (Col1-Thy-1) where Thy-1 inactivation is accomplished by Cre/loxP site-specific recombination to confirm that Thy-1 mediated apoptosis in fibroblasts is not an effect of total ablation of Thy-1. Staining of FasL, β3 integrin and Thy-1 in the granulation tissue demonstrated that these proteins were expressed in the same area of granulation tissue indicating a close co-localization of β3 integrin, FasL and Thy-1 expressing cells within the granulation tissue. Analysis of FasL expression in granulation tissue of healing skin wounds revealed that loss of Thy-1 expression in fibroblasts of Col1-Thy-1 mice was accompanied by decreased FasL expression compared to Thy-1 expressing control mice. Moreover, we could show that fibroblasts directly isolated from wounds of Col1-Thy-1 mice displayed significant less FasL expression and increased cell growth indicating that Thy-1 dependent FasL regulation is attributed to fibroblasts.

Here, we give first mechanistic insight into Thy-1 mediated control of the balance between proliferation and apoptosis of dermal fibroblasts.

P040

Decreased expression of CD49d in monocytes of ERp29$^{-/-}$ mice

U. Lippert[1], P. Hoyer[2] and D. M. Ferrari[3] [1]*Georg-August-University, Department of Dermatology and Venereology, 37075 Goettingen, Germany;* [2]*Institute of Transfusion Medicine, Red Cross Blood Transfusion Service NSTOB, 06847 Dessau, Germany;* [3]*Max-Planck Research Unit for Enzymology of Protein Folding, 06120 Halle/Saale, Germany*

Chaperones assist the folding of many secretory proteins in the endoplasmic reticulum (ER). The ER chaperone complement includes the lectin proteins calreticulin and calnexin, the ER Hsp70-related protein BiP, the Hsp90 homologue Grp94 and the Protein Disulfide Isomerase (PDI) family. These proteins constitute a quality control machinery for nascent polypeptide chains, ensuring proper folding and post-translational processing. The levels of many of these chaperones are regulated by stress conditions such as oxidative or metabolic stress. It has become increasingly clear in recent years that overloading and malfunction of the folding machinery can influence expression of secretory proteins such as cytokines or hormones since unfolded secretory proteins are generally retained and/or degraded by the ER associated degradation (ERAD) pathway. The proper function of ER chaperones is therefore a key mechanism for maintaining cell homeostasis and can influence important cell functions such as differentiation, survival and cell signaling pathways, involved in defense against pathogens and cancer. Although several reports exist about the role of Hsp70 and Hsp70-related proteins such as BiP, the physiological relevance of most PDI-related proteins remains far less clear. ERp29 is a two-domain PDI-related protein that lacks the PDI typical redox activity. Therefore, the function of ERp29 is redox independent and likely mainly based on chaperone activity. We have generated an ERp29-deficient mouse strain that to our knowledge constitutes the only full animal knockout of a PDI related protein to date. Using this model we were able to show that monocytes of peripheral blood express significantly less (11 $\pm$ 3%) of the alpha4 integrin (CD49d) compared to wild type mice (66 $\pm$ 19%) via flow cytometry analysis and double staining with CD115 as a marker for monocytes. CD49d is involved in the development of lymphocytes and migration of leukocytes into several tissues as well as in recruitment of mast cells into the gut and into the lung during inflammation. These findings indicate that ERp29 may have an important role in mediating cell migration via regulation of the folding of specific secretory proteins in the ER.

P041

Resveratrol: a novel anti-lymphangiogenic compound?

I. Hrgovic, A. Berndt, M. Doll, T. Hailemariam-Jahn, J. Kleemann, R. Kaufmann, S. Kippenberger and M. Meissner *Johann Wolfgang Goethe-University Hospital, Department of Dermatology, Venerology and Allergology, Frankfurt/Main*

Background: There is growing evidence that lymphatic vessels are linked to immune regulation, atherosclerosis, or metabolic diseases. In addition, the lymphatic vessels provide a route for tumor cells to metastasize. Therefore, influencing lymphangiogenesis is an interesting target in various pathological conditions. Signaling via the vascular endothelial growth factor receptor-2/-3 (VEGFR-2/3) and Tie-2 pathways is critical for lymphangiogenic responses. Recent studies suggest that Resveratrol, a natural phenol and phytoalexin found in the skin of red grapes, may mediate part of their antitumor effects by interfering with angiogenesis. Therefore, we explored whether the known anti-tumorigenic properties of Resveratrol might be additionally mediated in part by anti-lymphangiogenic effects through the reduction in VEGFR-2/3 and Tie-2 expressions in primary human lymphatic endothelial cells.

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

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

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

Chemokines/Cytokines

P042

In-depth characterization of the expression of IL-17 isoforms in psoriasis

L. Senra[1], R. Stalder[1], W. Boehncke[1,2] and N. C. Brembilla[1] [1]*University of Geneva, Department of Pathology and Immunology, 1211 Geneva, Switzerland;* [2]*University Hospitals of Geneva, Department of Dermatology and Venereology, 1211 Geneva, Switzerland*

Background: Psoriasis is a chronic recurrent inflammatory skin disease. Several lines of evidence point towards a central role for TNF-alpha, IL-23, and IL-17 in its pathogenesis, with strategies blocking IL-17A or the IL-17RA receptor subunit being so far the most effective way to treat this disease.

Objective: We undertook an in-depth analysis of the expression of IL-17 isoforms signaling via the IL-17RA subunit (IL-17A, IL-17F, IL-17C and IL-17E) in psoriasis.

Methods: Biopsies were taken from lesional (n = 10) and non-lesional psoritaic skin (n = 7), biopsies from normal human skin (n = 7) served as controls. The types of cells expressing IL-17 isoforms were assessed by immunohistological techniques and quantified by an automated imaging processing approach. In situ hybridization was used to determine *in vivo* transcription. Co-localization analysis were performed to dissect endocytosis /exocytosis pathways. Macrophages were generated by blood-dervied monocytes and tested for their ability to produce or internalize IL-17E. Levels of IL-17E mRNA and protein were measured by RT-PCR and western blot, respectively.

Results: IL-17E+ cells were increased in lesional psoriatic skin in addition to IL-17A + cells when compared to non lesional and normal skin. No differences in the number of IL-17F and IL-17C expressing cells were observed among the three study groups. In the epidermis, keratinocytes represented the major source of IL-17E, as revealed by their high expression of IL-17E mRNA *in vivo*. In line with this finding, keratinocytes extracted from lesional skin produced more IL-17E at steady state than those extracted from non-lesional skin of the same individual or unrelated normal skin. IL-17A+ neutrophils were also enriched in lesional epidermis. Most cells positive for IL-17A or IL-17E in the inflammatory infiltrate were located in the uppermost part of the papillary dermis, in immediate vicinity of the epidermis. Dermal IL-17E+ cells were mainly macrophages, which up-take IL-17E in a mechanisms dependent on clathrin and independent on caveolin. Consistently, monocyte-derived macrophages were unable to produce IL-17E mRNA *in vitro* while efficiently and time-dependently internalizing the cytokine. Few T cells, mast cells and neutrophils positive for IL-17E were observed. IL-17A positivity was found predominantly in dermal mast cells, which were capable of synthetizing IL-17A and store it in secretory granules. A minority of T cells, neutrophils and macrophages co-stained with IL-17A. The distribution of IL-17A and IL-17E expressing cells was similar in lesional and non lesional skin. Despite that, the absolute number of IL-17A+ mast cells and IL-17E+ macrophages were higher in lesional psoriatic skin, provided that mast cells and macrophages were significantly more abundant in lesions.

Conclusion: Our data suggest that IL-17E is primarily produced by psoriatic keratinocytes and is up-taken by dermal macrophages. In addition, we provide further evidence for mast cells being an important source of IL-17A in the dermis of psoriatic lesional skin. Together, we propose IL-17E as a new important player in the IL-17 mediated effects in psoriasis.

P043

IL-17AF signaling deficient mice show spontaneous infections with Staphylococcus aureus concomitant with expanded populations of $\gamma\delta$ T cells

S. Moos[1], A. P. Heinen[1], T. Regen[1], S. Voglis[1], K. El Malki[1], F. Wanke[1], I. Prinz[2], A. Diefenbach[3], A. Waisman[1] and F. C. Kurschus[1] [1]*Universitätsmedizin der Johannes Gutenberg-Universität Mainz, 55131 Mainz, Germany;* [2]*Institute of Immunology, Hannover, Germany;* [3]*Universitätsmedizin der Johannes Gutenberg-Universität Mainz, Institute of Immunology, 55131 Mainz, Germany*

Introduction: 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. The IL-17 response of TH17 cells and especially IL-17A-producing $\gamma\delta$; T cells are crucial for mice to fight infections with the coccal bacterium Staphylococcus aureus. IL-17F is the one member of the IL-17 family having the greatest homology to IL-17A. Moreover, IL-17A and IL-17F signal either as homo- or as heterodimers through a dimeric receptor composed of IL-17RA and IL-17RC.

Materials and Methods: We used previously described IL-17RA deficient mice (El Malki et al., 2013) and IL-17AF double deficient mice (Haas et al., 2012). Mice were aged and analyzed for spontaneous bacterial infections. Furthermore, we analyzed lymphoid organs and skin by flow cytometry, histology and gene expression by RTPCR

Results: IL-17RA deficient and IL-17AF double deficient mice older than 3 months spontaneously developed lesions in the cervical region and around mouth, ears and eyes. Microbiological analysis showed that these chronic infections are composed of Staphylococcus aureus. Associated with the bacterial infection, we found an expansion of RORγt expressing $\gamma\delta$ T cells in IL-17AF double deficient mice.

Conclusion: Mice deficient for IL-17 signaling were strongly susceptible to spontaneous Staphylococcus aureus infections. The respective Staphylococcus aureus strains and expanding $\gamma\delta$ T cell populations will be further characterized.

References: El Malki, K., et al. (2013). The Journal of investigative dermatology 133, 441–451.
Haas, J.D., et al. (2012). Immunity 37, 48–59.

P044

IL-17A disturbs skin barrier formation in 3D organotypic skin models

C. Pfaff[1,2], Y. Marquardt[1], K. Czaja[1], B. Lüscher[2] and J. M. Baron[1] [1]*RWTH Aachen University, Department of Dermatology, 52074 Aachen;* [2]*RWTH Aachen University, Institute of Biochemistry and Molecular biology, 52074 Aachen*

Psoriasis is one of the most prevalent autoimmune skin diseases. The proinflammatory cytokine IL-17A is found to be up-regulated in psoriatic lesions and is suggested to play a key role in the pathogenesis of the disease. Therefore we were interested to determine whether IL-17A affects the formation and the functionality of the skin barrier.

We treated organotypic 3D skin equivalents of human epidermal keratinocytes (NHEKs) with IL-17A and compared these to untreated models. For these experiments we used two different types of skin models. On the one hand we used psoriasis models developed with NHEKs and dermal fibroblasts from psoriatic lesions of patients and on the other hand control models containing cells from healthy donors. The IL-17A stimulation resulted in changes in skin morphology in both models including parakeratosis and varying epidermal thickness. Microarray analysis and immunohistological stainings of IL-17A treated control and psoriasis 3D models revealed down-regulation of genes and proteins important for epidermal differentiation and skin barrier formation, including filaggrin, involucrin and loricrin. In addition, an increased expression of different antimicrobial peptides (AMPs), including human beta defensins (hBDs) as well as members of the S100 calcium binding family could be detected. Furthermore a significant up-regulation of all members of the IL-36 cytokine family was measured in IL-17A treated 3D models. In summary we found that in both models the same gene clusters were deregulated upon IL-17A stimulation. However we observed qualitative differences as some of the IL-17A target genes were considerably stronger deregulated in the psoriasis model. This suggests that the psoriatic models are hypersensitive to IL-17A treatment. For example the expression of IL-36γ was markedly increased in 3D models from lesional keratinocytes and fibroblasts compared to models containing cells from healthy donors. Thus it is tempting to speculate that the IL-17A effects on skin cells were at least in part mediated by the induction of IL-36 cytokines in keratinocytes. Indeed their application was sufficient although to a lower extent compared to IL-17A to induce the expression of genes encoding different AMPs, including S100A7A, S100A12 and hBD-2, in NHEKs. Moreover we addressed which signaling pathways are relevant for the IL-17A effects. We found that the p38 MAPK as well as NF-κB pathways were necessary for the induction of the expression of IL-36α and AMPs in NHEKs. In further experiments we tested Secukinumab, an antibody that interferes with IL-17A function, for its ability to rescue the response to IL-17A treatment. We observed that Secukinumab was able to block the IL-17A induced deregulation of AMP and IL36 cytokine genes in NHEK monolayer cultures.

In conclusion we were able to establish 3D organotypic skin equivalents with NHEKs and fibroblasts of psoriasis patients. In these as well as in control models IL-17A affected differentiation with the psoriatic models being more susceptible to the treatment. The analysis of the downstream consequences suggests that IL-36 family members are important to mediate the IL-17A effects.

P045

Immunomodulatory potential of starPEG heparin hydrogels on wound healing associated chemokines

N. Lohmann[1,2], L. Schirmer[2,3], E. Wandel[1,2], P. Atallah[2,3], I. Forstreuter[1,2], R. A. Ferrer[1,2], U. Freudenberg[2,3], C. Werner[2,3], J. C. Simon[1,2] and S. Franz[1,2] *[1]Department of Dermatology, Venerology and Allergology, Leipzig University, Leipzig; [2]Collaborative Research Center (SFB-TRR67) Matrixengineering, Leipzig and Dresden; [3]Leibnitz Institute of Polymer Research Dresden, Max Bergmann Center of Biomaterials Dresden, Center for Regenerative Therapies Dresden, Dresden*

Incidences of non-healing wounds are constantly rising and significantly contribute to morbidity and mortality worldwide. Non-healing wounds do not progress the phases of normal wound repair but are stuck in a chronic inflammatory response. The wound healing process is governed by a wide range of inflammatory and regenerative signaling molecules. In non-healing wounds the balance of these signals is shifted towards an excess of inflammatory cytokines leading to continuing infiltration of immune cells which release more inflammatory cytokines and promote tissue breakdown. Resolution of this unrestrained inflammation loop represents an unmet challenge in the treatment of non-healing wounds. Here, we suggest biohybrid starshaped polyethylene glycol (starPEG) heparin hydrogels as sequestration matrix which binds inflammatory cytokines overproduced in chronic wounds and thus help to restore a healthy wound environment.

To determine the hydrogels' sequestration profiles for inflammatory cytokines binding assays with recombinant mediators and supernatants (SN) from activated dermal fibroblast (dFb) or inflammatory M1 macrophages were performed. In all conditions abundant binding of the chemotactic factors MCP-1 and IL-8 was observed whereas the cytokines IL-1b, TNF and IL-6 were not targeted by the hydrogels. The impact of MCP-1 and IL-8 sequestration on their function as chemoattractant was investigated in a transmigration assay with primary human monocytes and polymorphonuclear cells (PMN). SN derived from activated dFb and r.h. chemokines after incubation with the hydrogels were used as chemotactic stimuli. Indeed, depletion of MCP-1 and IL-8 by the hydrogels decreased migration of monocytes and neutrophils, demonstrating the functional neutralization of these mediators by the hydrogels. Applying these hydrogels in a complex *in vivo* situation using a wound healing model of full thickness excisional wounds in mice confirmed their abundant sequestration activity. Chemokines from the wound environment could be detected in the hydrogel networks. Infiltration of monocytes and PMN was quantified in digested wound biopsies by flow cytometry. Strikingly, influx of neutrophils and monocytes/macrophages were significantly decreased in wounds after application of the hydrogels for 5 days. No haemorrhagic effects were observed.

In conclusion, starPEG heparin hydrogels could be of value as immunomodulating wound dressing supporting inflammatory resolution through the sequestration and neutralization of chemokines and consequential reduction of immune cell infiltration.

P046 (O01/04)

CXCL16 enhances migratory properties of neutrophils in psoriasis

S. Bach[1], S. Abraham[1], M. Herbig[2], S. Meisterfeld[1], K. Blau[1], N. Zimmermann[1], J. Guck[2] and C. Günther[1] *[1]Technical University Dresden, Department of Dermatology, University Hospital, Dresden, Germany; [2]Technical University Dresden, Biotechnology Center, Dresden, Germany*

Psoriasis is a chronic inflammatory skin disease characterized by infiltrating immune cells. Their recruitment into skin is in great part orchestrated by a network of chemokines. We showed that the chemokine CXCL16 is upregulated in psoriatic skin and contributed to skin homing of CXCR6+ CD8+ T cells. CXCL16 expression in psoriasis is induced by TNFalpha and TLR2 or TLR7 stimulation of antigen presenting cells as well as keratinocytes. CXCL16 exerts its function by ligation of its receptor CXCR6. We found that in addition to T cells also neutrophils of psoriatic patients express CXCR6. As neutrophils are one of the first cells recruited into psoriatic lesions and exert proinflammatory functions by IL-17 production as well as oxidative burst we were interested in investigating the effects of CXCL16 on this proinflammatory cell population. *In vitro* migration assays demonstrated that CXCL16 could induce migration of neutrophils. Simultaneous stimulation by CXCL16 and CXCL8/IL-8, another important neutrophil chemoattractant, resulted in an enhanced migratory response of neutrophilic granulocytes. This highlights the importance of cumulative effects in the chemokine network in psoriasis.

Migration inside the tissue requires active mechanical deformation of neutrophils. Using real time deformability cytometry we analysed cell deformability at rates of 100 cells/s, approaching the throughput of conventional flow cytometers. We could show that CXCL16 induces mechanical and morphological changes compared to untreated neutrophils. The softening of the cells and a noncircular cell shape likely favors neutrophil migration inside the tissue.

In conclusion we have demonstrated CXCL16 upregulation in psoriasis that mediated neutrophil migration and thereby enhanced the chemotactic response induced by CXCL8. In addition, CXCL16 resulted in increased mechanical deformability of neutrophils enabling their transmigration into tissue. The exploration of this new pathway for neutrophil recruitment may also lead to future evaluation of CXCL16 as a potential target for therapeutic intervention in psoriasis.

P047

Antineoplastic modulation of the cutaneous micromilieu by ingenol mebutate

J. Baran, S. A. Braun, H. Schrumpf, B. Homey and P. A. Gerber *Heinrich-Heine University, Department of Dermatology, 40225 Düsseldorf, Germany*

Introduction: Ingenol mebutate (IM) is a first-in-class macrocyclic diterpene ester, which is approved for the treatment of non-hyperkeratotic actinic keratosis. The mode of action is still not completely understood. A dual mechanism of action is proposed: (i) induction of rapid cell necrosis and apoptosis in high concentrations of the active substance and (ii) a specific immunologic response, which recruits a neutrophil-rich inflammatory infiltrate.

Objective: To further elucidate the specific mode of action of IM.

Methods: Human keratinocytes and epithelial tumor cell lines were treated with different concentrations of ingenol mebutate. mRNA expression and protein levels of selected chemokines were analyzed by qPCR and ELISA-assays of supernatants.

Results: IM induces a specific upregulation of certain inflammatory chemokines. Notably, chemokine induction was induced at significantly higher levels in certain tumor cell lines as compared to primary keratinocytes.

Conclusion: Our results support the hypothesis that the effects of IM in the management of actinic field cancerization are indeed mediated by a 'tumor cell selective' immune response and are not only the result of an unspecific toxic reaction. The molecular mechanisms of IM-induced chemokine expression in healthy keratinocytes as compared to neoplastic cells remain to be elucidated.

P048 (O02/03)

Deciphering the role of IL-36 in psoriasis

E. Witte[1], W. Döcke[2], M. Ramm[1], N. Schmidt[2], S. Jodl[2], H. Volk[3,4], W. Sterry[5], K. Wolk[1,3] and R. Sabat[1,6] *[1]University Hospital Charité, Psoriasis Research and Treatment Center, 10117 Berlin, Germany; [2]Bayer Pharma AG, Global Drug Discovery, 13353 Berlin, Germany; [3]University Hospital Charité, Berlin-Brandenburg Center for Regenerative Therapies, 10117 Berlin, Germany; [4]University Hospital Charité, Institute of Medical Immunology, 10117 Berlin, Germany; [5]University Hospital Charité, Department of Dermatology and Allergy, 10117 Berlin, Germany; [6]University Hospital Charité, Research Center Immunosciences, 10117 Berlin, Germany*

Approaches that modulate the action of cytokines have a high therapeutic potential in inflammatory diseases such as psoriasis. To better understand the inflammatory cascades in psoriasis, we individually quantified the gene expression levels of 35 cytokines in psoriatic lesions in comparison to healthy skin. IL-36alpha, a new member of the IL-1 cytokine family, showed the strongest upregulation. Subsequent analyses revealed that also other members of the IL-36 family, namely IL-36beta and IL-36gamma, were elevated in psoriasis lesions.

In order to unravel the effects of IL-36 in psoriasis pathogenesis, we performed whole mRNA deep sequencing analysis of immune cells, endothelial cells, fibroblasts, and keratinocytes, which had been stimulated with IL-36beta. Interestingly, the highest number of regulated gene expressions was observed in fibroblasts, followed by keratinocytes. In both cell types, IL-36 increased the expression of many molecules known to support infiltration of immune cells into the skin. *In vitro* experiments confirmed the inducing effect of IL-36 on selected chemokines including CCL20 and CXCL1 in fibroblasts and keratinocytes. Accordingly, in psoriatic lesions IL-36 levels positively correlated with the levels of CCL20 and CXCL1. Furthermore, IL-36 additionally strengthens the expression of CD54, a molecule enabling cell-cell interaction and immune cell migration along tissue cells.

Beside these skin infiltration-promoting molecules, IL-36 induced several interleukins in fibroblasts and keratinocytes. These included those with documented role in psoriasis pathogenesis (like IL-24) as well as relatively unknown mediators (like IL-32), whose biology we are addressing in further experiments. Finally, IL-36 elevated the expression of anti-microbial proteins like lipocallin 2 (LCN2) in keratinocytes. Accordingly, LCN2 levels showed strong positive correlation with IL-36 expression in psoriatic lesions, and LCN2 blood concentrations were increased in psoriasis patients compared to healthy donors.

Regarding the sources of IL-36 cytokines in psoriasis lesions, we found that these cytokines were produced by different cell populations. For example, IL-36gamma was dominantly expressed by keratinocytes stimulated with IL-22 or IL-36beta. Accordingly, strong positive correlations between IL-36gamma expression on the one side and the levels of IL-22 and other IL-36 cytokines on the other side were detected in psoriatic lesions.

In summary, our results suggest that in psoriatic skin the dominantly present IL-36 cytokines are involved in a cascade that comprises TNF-alpha, IL-17, IL-22, IL-36, IL-24, IL-32, LCN2, CD54 and chemokines, mainly affect fibroblasts and keratinocytes, and lead to skin infiltration of immune cells and epidermis alterations.

Clinical Research

P049

Active and passive antimicrobial wound dressings exerting an antibacterial effect on Pseudomonas aeruginosa and a Staphylococcus aureus biofilm *in vitro*

C. Wiegand[1], K. Reddersen[1], M. Abel[2], P. Ruth[2], S. de Lange[2], J. Muldoon[3] and U. Hipler[1] *[1]Universitätsklinikum Jena, Jena, Germany; [2]Lohmann & Rauscher GmbH & Co. KG, Rengsdorf, Germany; [3]Activa Healthcare, Burton-upon-Trent, United Kingdom*

Introduction: An increased bacterial load on the surface of a wound amplifies and/ or perpetuates a pro-inflammatory environment. It has been suggested, that a lower probability of healing is seen when four or more pathogens are present, based on their synergistic relationship. It is now widely accepted that them forming biofilms, complex structures consisting of bacteria cells embedded in an extracellular matrix consisting of hydrated extrapolymeric substances (EPS), further lowers the probability of healing. Hence, it was postulated that it is necessary to create conditions that are unfavorable to micro-organisms and favorable for the host repair mechanisms. Wound dressings featuring active antimicrobial agents or a passive antimicrobial mechanism may help in the treatment of chronic wounds.

Methods: The dressings Cutimed® Sorbact® dressing pad (BSN medical), Vliwasorb® (Lohmann & Rauscher), Vliwaktiv® Ag (Lohmann & Rauscher), and Suprasorb® X+PHMB (Lohmann & Rauscher) were investigated. According to the JIS L 1902:2002, samples of 400 mg of the dressings were used for testing. The samples were incubated up to 24 h at 37°C under aerobic conditions with P. aeruginosa growth. Furthermore, a S. aureus biofilm was cultivated on glass plates, covered with the dressings, and incubated for 24 h at 37°C. Then, dressings were removed and glass plates further incubated for 48 h. Biofilm on the glass plates was evaluated directly after dressing removal and following 48 h re-growth period using the fluorescent alamar blue assay.

Results: The dressings Cutimed® Sorbact® dressing pad, Vliwasorb®, Vliwaktiv® Ag, and Suprasorb® X+PHMB displayed a complete inhibition of Pseudomonas aeruginosa growth. The antibacterial effect achieved against Pseudomonas aeruginosa could be rated as strong antibacterial activity according to JIS L 1902:2002 (log-reduction >3). Furthermore, it was found that treatment of the Staphylococcus aureus biofilm with the dressings efficiently reduced biomass *in vitro*. Significantly less viable bacteria were observed after incubation with Cutimed® Sorbact® dressing pad, Vliwasorb®, Vliwaktiv® Ag, and Suprasorb® X+PHMB. However, only Suprasorb® X+PHMB exhibited a remanescent effect and was able to inhibit biofilm re-growth over a time period of 48 h.

Conclusions: It could be shown that antimicrobial dressings can decrease multiplication of bacteria by passive mechanisms based on securely binding the microbes in or to the dressing as observed for the DACC-coated dressing Cutimed® Sorbact® or the SAP-containing dressing Vliwasorb®. However, dressings that actively release antimicrobial agents like silver ions or PHMB are thought to have an additional effect reaching bacteria beyond direct contact to the dressing. Here, PHMB was found to be superior to Ag+ demonstrating a remanescent effect and preventing biofilm re-growth *in vitro*.

P050

Effect of non-adhering dressings on promotion of fibroblast proliferation and wound healing *in vitro*

C. Wiegand[1], D. Winter[1], M. Abel[2], P. Ruth[2], K. Grießhammer[2] and U. Hipler[1] [1]*Universitätsklinikum Jena, Jena;* [2]*Lohmann & Rauscher GmbH & Co. KG, Rengsdorf*

Introduction: Dressings can stick to the wound surface due to dried drainage, ingrowths of newly formed tissue or a clammy dressing surface. This adhesion can cause problems since dressing removal will disrupt the wound bed and destroy newly formed, healthy tissue. Wound contact dressings are non-adhering dressings that are most commonly used during the phase of granulation, tissue formation, and re-epithelialisation. However, any dressing that is applied to a wound comes into intimate contact with cells involved in the healing process. Determination of the effects of different non-adhering wound dressings on cell viability and proliferation during wound healing but also on their migration capacity is consequently of interest. Cell reactions may also be accompanied by changes in cell morphology and structure, where cytotoxic effects lead to the loss of actin and tubulin networks while positive signals could result in an improved expression of these cell structure proteins.
Methods: The non-adhering dressings Lomatuell® Pro (Lohmann & Rauscher), UrgoTül® (URGO), Atrauman® Impregnated dressing (HARTMANN), and Hydrotüll® (HARTMANN) were investigated. Wound dressing samples were cut aseptically corresponding to 1.5 cm × 1.5 cm and specimens were either used directly for testing or were extracted prior to testing. The number of viable, metabolically active cells was determined using the photometric MTT assay. Determination of cell proliferation was carried out using a luminometric ATP assay. For evaluation of cell morphology and structure, the cell nucleus was stained using DAPI, F-actin was dyed with MFP TM-DY-549P1-Phalloidin, and tubulin was detected using an anti-alpha-tubulin monoclonal antibody and Alexa Fluor® 488 goat anti-mouse IgG (H+L). NHDF monolayers were scratched with a sterile pipette tip and wound dressing samples were placed directly on the scratch to be incubated for 4, 24, 48, and 144 h. After the respective incubation periods, cells were stained with hematoxylin and eosin. Microscopic evaluation was carried out using the Axio Scope A.1 (Carl Zeiss GmbH) and images were obtained with the digital camera ColorView II (Soft Imaging Systems).
Results: It could be shown that the non-adhering dressings Lomatuell® Pro and UrgoTül® do not negatively affect NHDF *in vitro*. During treatment with these dressings, the cells demonstrated good viability as well as normal cell morphology and proliferation. In contrast, the products Hydrotüll® and Atrauman® noticeably decreased cell viability and proliferation in this study. In accordance, treatment with these dressings led to loss of normal cell morphology. Furthermore, it was demonstrated that Lomatuell® Pro and UrgoTül® exhibit no harmful effects on scratch wound healing *in vitro*. In contrast, the products Hydrotüll® and Atrauman® noticeably decreased the healing progression and the scratches remained open.
Conclusions: Here, a comprehensive *in vitro* approach was used to evaluate possible effects of non-adhering wound contact dressings used during the phase of granulation, tissue formation, and re-epithelialisation. Results clearly showed that different outcomes can be expected. It was observed that non-adhering dressings like Lomatuell® Pro can prevent damage to newly formed tissue and might thereby positively influence the wound healing outcome.

P051

In-vitro-assessment of fluid management by PU foam dressings under compression using a vertical maceration model

C. Wiegand[1], K. Reddersen[1], M. Abel[2], P. Ruth[2], J. Muldoon[3] and U. Hipler[1] [1]*Universitätsklinikum Jena, Jena, Germany;* [2]*Lohmann & Rauscher GmbH & Co. KG, Rengsdorf, Germany;* [3]*Activa Healthcare, Burton-upon-Trent, United Kingdom*

Introduction: Maceration is the elixation of the skin by prolonged exposure to moisture that impedes healing due to failure of the skin protection and possible microbial infections. Modern wound dressings are expected to maintain a humid wound milieu without allowing exposure of the peri-wound skin to exudate and subsequent damage of the skin by maceration. Hence, it is of interest to analyze and compare the fluid management of PU-foam dressings under standardized conditions as close as possible to a real life situation. Therefore, a vertical maceration model using 40 mmHg compression was developed.
Methods: The PU foam dressings Suprasorb® P 7.5 × 7.5 cm (Lohmann & Rauscher); ALLEVYN® Gentle 10 × 10 cm (Smith & Nephew), ALLEVYN® non-adhesive 9 × 11 cm (Smith & Nephew), ALLEVYN® LIFE 10.3 × 10.3 cm (Smith & Nephew), Mepilex® Border 10 × 10 cm (Mölnlycke Health Care), and Mepilex® Non-Border 10 × 12 cm (Mölnlycke Health Care) were investigated. They were applied to an artificial wound in a gelatine-based tissue substitute for the vertical maceration test under 400 mmHg compression. Evaluation of fluid uptake and distribution in the dressings was performed by video recording. In addition, shape loss of the dressings, maximal fluid uptake and time to maceration was determined.
Results: The dressings Mepilex® Non-Border, ALLEVYN® Gentle, Suprasorb® P and ALLEVYN® non-adhesive displayed a distinctly higher fluid absorption capacity (FAC) compared to Mepilex® Border and ALLEVYN® LIFE. It could be shown that Suprasorb® P and ALLEVYN® LIFE demonstrated a similar FAC per [g] before maceration occurred that was significantly higher compared to the remaining PUfoam dressings. Furthermore, Suprasorb® P displayed the best form stability in the tests. In contrast, ALLEVYN® LIFE exhibited a significant expansion while only slight changes were observed for ALLEVYN® Gentle, ALLEVYN® non-adhesive, ALLEVYN® LIFE, Mepilex® Border, and while Mepilex® Non-Border *in vitro*.
Conclusions: In conclusion, the *in vitro* maceration model was successfully applied in a vertical position to quantify and evaluate differences between PU-foam wound dressings with regard to fluid management under simulated compression.

P052

Efficacy of ixekizumab in patients with plaque psoriasis, with and without previous exposure to biologic therapies: results at weeks 12 and 60 from UNCOVER-1

S. Gerdes[1], N. Korman[2], S. Wilhelm[3], M. Casillas[3], F. Zhao[3], A. Schacht[3] and Y. Dutronc[3] [1]*University Medical Center Schleswig-Holstein Campus Kiel, Department of Dermatology, 24105 Kiel, Germany;* [2]*University Hospitals Case Medical Center, Murdough Family Center for Psoriasis, 3500 Cleveland, USA;* [3]*Eli Lilly and Company, Lilly Corporate Center, 46285 Indianapolis, USA*

Introduction & Objectives: Ixekizumab is an anti-IL-17A monoclonal antibody that has been studied in a phase 3 randomized, double-blind, placebo controlled trial of patients with psoriasis. The objective of this subgroup analysis was to evaluate the efficacy of ixekizumab compared to placebo in patients with moderate-to-severe plaque psoriasis with or without previous exposure to biologic therapy at weeks 12 and 60.

Materials & Methods: In this study, 1296 patients were randomized to receive subcutaneous placebo ($N = 431$), or a single injection of 80 mg ixekizumab once every 2 (IXE Q2W, $N = 432$) or 4 weeks (IXE Q4W, $N = 433$) following a 160 mg starting dose at Week 0. At week 12, patients with a static physician global assessment (sPGA) of 0 or 1 were re-randomized to receive IXE Q4W ($N = 229$), IXE Q12W ($N = 227$) or placebo ($N = 226$) until week 60 or relapse.. Efficacy was evaluated by the proportion of patients with ≥75% improvement in Psoriasis Area and Severity Index (PASI 75), the proportion of patients with 100% improvement in PASI (PASI 100) and the proportion of patients with sPGA 0 or 1 in subgroups of patients who had previous exposure to biologics and those who were naïve to biologic therapy. Treatment groups were compared using Fisher's exact test within each subgroup and missing values were imputed as non-response.
Results: In this analysis, 522 patients had received prior biologic treatment, and 774 were naïve to biologic therapy. In these subgroups, PASI 75 response rates at Week 12 were 87.9% and 90.0% for IXE Q2W, and 78.6% and 85.2% for IXE Q4W, respectively, each significantly greater than those for placebo (3.3% and 4.4%, respectively, $P < 0.001$). Similarly, the sPGA 0/1 response rates at Week 12 were 78.6% and 83.8% for IXE Q2W and 67.3% and 82.2% for IXE Q4W, respectively, each significantly greater than those for placebo (2.2% and 4.0%, respectively, $P < 0.001$). In the same subgroups, the proportion of patients who achieved complete resolution of psoriasis (PASI 100) at week 12 were also significantly higher in the IXE Q2W (34.7% and 35.8%, respectively) and IXE Q4W (33.9% and 33.3%, respectively) compared to placebo (0 and 0, respectively, $P < 0.001$).
Among patients achieving sPGA 0/1 at Week 12 who received continued dosing on the IXE Q4W regimen (Weeks 12–60), 50.4% of biologic experienced and 53.4% of biologic naïve attained or maintained PASI 100 at Week 60.
Conclusions: Ixekizumab has shown high levels of response in the treatment of patients with moderate-to-severe psoriasis irrespectively of previous exposure to biologic therapy, both in short term (week 12) and long term treatment (week 60).

P053

The impact of ixekizumab treatment on health-related quality of life in patients with moderate-to-severe psoriasis: results from UNCOVER-1

M. Augustin[1], K. Gordon[2], E. Nikai[3], E. Edson-Heredia[3], B. Zhu[3], O. M. Goldblum[3], H. Carlier[3] and R. G. Langley[4] [1]*University Hospital Hamburg-Eppendorf, Institute for Healthcare Research in Dermatology and the Care Professions (IVDP), 20246 Hamburg, Germany;* [2]*Northwestern University Feinberg School of Medicine, Department of Dermatology, 60611 Chicago, USA;* [3]*Eli Lilly and Company, Lilly Corporate Center, 46285 Indianapolis, USA;* [4]*Dalhousie University, Department of Medicine, B3H 4R2 Halifax, Canada*

Background & Objective: Psoriasis (Ps) can greatly affect patient's health-related quality of life (HRQoL). The objective of this study was to evaluate changes in patients' HRQoL during treatment for Ps with ixekizumab, an anti-IL-17A IgG4 monoclonal antibody with high binding affinity, compared with placebo over 12 weeks.
Methods: In this Phase 3, multicenter, double-blind trial, 1296 patients were randomized to receive subcutaneous placebo (PBO; $N = 431$) or 80-mg ixekizumab as one injection every 2 weeks (IXE Q2W; $N = 433$) or every 4 weeks (IXE Q4W; $N = 432$), for a duration of 12 weeks, following a 160-mg starting dose at Week (wk) 0. Skin-based HRQoL was assessed by the Dermatology Life Quality Index (DLQI) at baseline (wk 0), wk 2, 4 and 12 (score range of 0–30; higher scores indicate worse HRQoL; score of 0.1 indicate 'no impairment in HRQoL'). Levels of function and health were assessed by the Short-Form 36 item (SF-36) Physical Component Summary (PCS) and Mental Component Summary (MCS) at baseline and wk 12 (score range of 0–100; higher scores indicate better levels of function and/or health). Treatment comparisons were made using analysis of covariance (ANCOVA) for continuous variables (after missing data was imputed using the last observation carried forward method) and logistic models for categorical variables.
Results: DLQI total mean scores at baseline were 13.2, 13.4 and 12.8 for IXE Q4W, IXE Q2W and PBO, respectively. Significant improvements in HRQoL were reported by patients treated with IXE Q4W and IXE Q2W as early as week 2, with a DLQI total mean score of 5.5 for both groups, compared with 11.3 for PBO at this timepoint. DLQI least squares [LS] mean changes from baseline at week 2 were −7.7 for IXE Q4W ($P < 0.001$ vs. PBO) and −7.8 for IXE Q2W ($P < 0.001$ vs. PBO) compared with −1.6 for PBO. Further improvements in HRQoL were reported at week 12, with DLQI total mean scores of 2.3 for IXE Q4W and 2.0 for IXE Q2W, compared with 11.6 for PBO; DLQI LS mean changes from baseline were −10.3 for IXE Q4W ($P < 0.001$ vs. PBO) and −10.7 ($P < 0.001$ vs. PBO) for IXE Q2W, compared with −0.7 for PBO. By wk 2, a significantly higher proportion of patients treated with ixekizumab compared with PBO experienced 'no impairment of HRQoL' because of Ps, as reported by a DLQI score of 0.1: n (%) of patients was 93 (21.5%) for both IXE Q4W and IXE Q2W groups, compared with 16 (3.7%) for PBO ($P < 0.001$). This proportion continued to be significantly higher at wk 12: n (%) 258 (59.7%) [$P < 0.001$ vs. PBO] for IXE Q4W and 287 (66.3%) [$P < 0.001$ vs. PBO] for IXE Q2W, compared with 20 (4.6%) for PBO. Improved SF-36 scores were also recorded at week 12, with a PCS LS mean change from baseline of 4.3 for both IXE Q4W and IXE Q2W, compared with −0.2 for PBO ($P < 0.001$), and MCS LS mean changes from baseline of 3.7 ($P < 0.001$ vs. PBO) and 4.1 ($P < 0.001$ vs. PBO) for IXE Q4W and IXE Q2W respectively, compared with 0.9 for PBO.
Conclusions: Treatment with both IXE Q4W and IXE Q2W was associated with significant improvements in HRQoL for patients with moderate-to-severe Ps. Furthermore, the improvements in HRQoL occurred as early as wk 2, the first post-baseline assessment, after beginning ixekizumab treatment, and a significant proportion of patients reported DLQI 0.1, indicating no impact of Ps on HRQoL.

P054

Impact of ixekizumab treatment on fingernail psoriasis: results from UNCOVER-1

L. Guenther[1,2], M. Sebastian[3], J. J. Wu[4], O. M. Goldblum[5], F. Zhao[5], S. Rathmann[5] and P. Rich[6] [1]*University of Western Ontario, Department of Medicine-Division of Dermatology, N6A 3K7 London, Canada;* [2]*Guenther Research Inc., N6A 3H7 London, Canada;* [3]*Hautarztpraxis Mahlow, 15831 Mahlow, Germany;* [4]*Kaiser Permanente, Los Angeles Medical Center, 90027 Los Angeles, USA;* [5]*Eli Lilly and Company, Lilly Corporate Center, 46285 Indianapolis, USA;* [6]*Oregon Health Science University, Dermatology and Clinical Research, 97239 Portland, USA*

Introduction & Objectives: Psoriasis affecting the fingernails can be difficult to treat. Ixekizumab, an anti-IL-17A monoclonal antibody, has been studied in a Phase 3, randomized, double-blind, placebo-controlled trial in moderate-to-severe psoriasis. The objective of this analysis was to determine the effect of ixekizumab on fingernail psoriasis in patients enrolled in a Phase 3, double-blind trial.
Materials & Methods: In this Phase 3, multicenter, double-blind trial, 1296 patients were randomized to receive subcutaneous placebo (PBO; $N = 431$) or 80-mg ixekizumab as one injection every 2 (IXE Q2W; $N = 433$) or every 4 weeks (IXE Q4W; $N = 432$), for a duration of 12 weeks, following a 160-mg starting dose at Week 0. At Week 12, ixekizumab-treated responders (patients with an sPGA score of 0 or 1) were re-randomized to receive PBO, 80 mg IXE Q4W, or 80 mg IXE every 12 weeks (IXE Q12W). All Week 12 nonresponders (patients with sPGA >1) received IXE Q4W from Weeks 12–60. Placebo-treated nonresponders received 160-mg IXE at Week 12, followed by IXE Q4W through Week 60. In patients with baseline psoriatic fingernails (PBO, $n = 283$; IXE Q2W, $n = 284$; IXE Q4W, $n = 283$), the Nail Psoriasis Severity Index (NAPSI) was used to assess fingernail severity. NAPSI scores range from 0 (no nail psoriasis) to 80 (severe nail psoriasis). Least squares (LS) mean changes and standard error were calculated using mixed effects models for repeated measures.

Results: Mean (±SD) baseline NAPSI was 25.0 ± 19.2 for patients with psoriatic fingernails. At Week 12, significant improvements from baseline NAPSI were observed in the IXE Q2W (7.2 ± 0.7) and IXE Q4W groups (7.2 ± 0.7) relative to PBO (2.2 ± 0.7, $P < 0.001$ each comparison). At Week 60, NAPSI scores were significantly improved from baseline for ixekizumab-treated responders re-randomized to IXE Q4W (19.3 ± 1.0) and IXE Q12W (12.0 ± 1.1) compared to PBO (5.8 ± 1.8, $P \leq 0.003$ each comparison). Additionally, at Week 60, placebo-treated nonresponders who switched to IXE Q4W at Week 12 experienced a 20.3 ± 18.9 mean (±SD) NAPSI reduction from baseline. The percentage of patients who experienced no nail psoriasis (NAPSI = 0) at 60 weeks was significantly higher among ixekizumab-treated responders randomized to IXE Q4W (46.8%) and IXE Q12W (23.1%) compared to PBO (1.9%, $P < 0.001$ each comparison).
Conclusions: Significant improvement in fingernail psoriasis was observed by Week 12 in patients treated with ixekizumab relative to PBO. Patients administered ixekizumab for the 60-week study duration demonstrated significant and sustained improvement in nail psoriasis, and a higher percentage had no nail psoriasis relative to those who were randomized to PBO. At 60 weeks, placebo-treated patients who switched to IXE Q4W at Week 12 experienced improvements comparable to the ixekizumab-treated responders who had extended treatment on Q4W.

P055

Safety and tolerability of ixekizumab: analysis of neutropenia in 7 clinical studies of moderate-to-severe plaque psoriasis

B. Strober[1], B. A. Pangallo[2], M. F. Veenhuizen[2], W. Xu[2], J. M. Russell[2] and A. Blauvelt[3] *[1]University of Connecticut, Health Center Farmington, 06030 Farmington, USA; [2]Eli Lilly and Company, Lilly Corporate Center, 46285 Indianapolis, USA; [3]Oregon Medical Research Center, 97223 Portland, USA*

Introduction & Objective: Ixekizumab is an anti-IL-17A IgG4 monoclonal antibody with high binding affinity being developed for the treatment of plaque psoriasis. IL-17A is known to play a role in mobilizing neutrophils. In this analysis, we assessed neutropenia and potential neutropenia-related treatment-emergent adverse events (TEAEs) in patients with moderate-to-severe plaque psoriasis treated with ixekizumab.
Materials & Methods: Hematologic and TEAE data were integrated using data from the induction period of 3 randomized, controlled trials (RCTs; 0–12 weeks [wks]), the maintenance period of 2 of the 3 RCTs with a randomized withdrawal design (12–60 wks), and all patients exposed to ixekizumab from all psoriasis trials (7 total trials controlled/uncontrolled). The induction period analyses included patients randomized to ixekizumab every 2 (IXE Q2W; $N = 1167$) or 4 wks (IXE Q4W; $N = 1161$) following a 160-mg starting dose, etanercept (50 mg biweekly; $N = 739$), or placebo ($N = 791$). The maintenance period included patients re-randomized to IXE Q4W ($N = 416$), ixekizumab every 12 wks (IXE Q12W, $N = 408$), or placebo ($N = 402$). The group of all patients exposed to ixekizumab ($N = 4204$) accounted for 4730 patient years of exposure. Neutropenia was assessed by National Cancer Institute-Common Terminology Criteria for Adverse Events: grade 1: < the lower limit of normal to ≥1.5 × 109/l; grade 2: <1.5 to ≥1.0 × 109/l; grade 3: <1.0 to ≥0.5 × 109/l; grade 4: <0.5 × 109/l.
Results: Among all patients exposed to ixekizumab, 2 patients discontinued due to neutropenia and no serious AEs were related to neutropenia. During the induction period, grade 2 neutropenia was observed in 2.1%, 1.9%, 3.3% and 0.3% of patients and grade 3 was uncommon occurring in 0.2%, 0, 0.5% and 0.1%, of IXE Q2W, IXE Q4W, etanercept and placebo groups, respectively. There was 1 case of grade 4 neutropenia in the IXE Q4W cohort; however, this patient was within normal range 2 days later. In maintenance, grade 2 neutropenia was noted in 1.9% and 1.2% of IXE Q4W and IXE Q12W, respectively, and 1.2% of placebo patients; grade 3 neutropenia was observed in 1 patient receiving IXE Q12W. Among all patients exposed to ixekizumab, grade 2 neutropenia was observed in 2.8% of patients, grade 3 in 0.2% of patients, and grade 4 in 2 patients (the IXE Q4W patient mentioned above and another patient in a long-term extension period who later returned to grade 2 on treatment). Neutrophil changes were transient, with counts recovering after continuous ixekizumab treatment. Only 1 patient had an infection (nasopharyngitis) with an onset date ≤14 days before grade 3 neutropenia was noted.
Conclusions: In patients treated with ixekizumab, low-grade neutropenia was uncommon and grade 3 or worse neutropenia was rare. Generally, neutropenia was transient, not associated with infection, and did not require discontinuation of ixekizumab.

P056

Validation of serological diagnostics in the detection of IgG autoantibodies against human collagen VII in epidermolysis bullosa acquisita (EBA) – a multicenter analysis

M. Hoch[1], S. Lotfi[1], G. Di Zenzo[2], A. Marzano[3], M. Goebeler[4], I. Lakos Jukic[5], J. Kern[6], C. Sitaru[6], M. Sardy[7], S. Uzun[8], H. Jedlikova[9], R. Gläser[10], E. Cozzani[11], T. Schmidt[1], R. Eming[1], G. Göpel[1], B. Greene[12], T. Hashimoto[13] and M. Hertl[1] *[1]Department of Dermatology, Marburg; [2]Istituto Dermopatico dell'Immacolata, Rome; [3]Dermatology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore, Milan; [4]Department of Dermatology, Wuerzburg; [5]Department of Dermatology, Zagreb; [6]Department of Dermatology, Freiburg; [7]Department of Dermatology, Munich; [8]Department of Dermatology, Antalya; [9]Department of Dermatology, Breno; [10]Department of Dermatology, Kiel; [11]IRCCS AOU San Martino Di.S.Sal., Genoa; [12]Institute of Biometry and Statistics, Marburg; [13]Department of Dermatology, Kurume*

EBA is a severe and often devastating autoimmune bullous skin disorder which affects skin and mucous membranes and is frequently associated with pronounced skin fragility and secondary scarring. Here, we aimed at comparing the sensitivity and specificity of four diagnostic procedures in the detection of serum IgG autoantibodies against human collagen VII (Col VII), the autoantigen of EBA. Sera from 98 patients with EBA, 100 patients with bullous pemphigoid (BP), 49 patients with pemphigus (PV/PF) and 50 age-matched healthy controls were analyzed by 1) indirect immunofluorescence (IF) with saline-split human skin (SSS), 2) immunoblot with recombinant human Col VII, 3) MBL Col VII-ELISA and 4) Euroimmun Col VII ELISA. Diagnosis of EBA was based on the characteristic clinical phenotype, direct and indirect IF or Col VII ELISA. Of the 98 studied EBA sera, 89 were positive by MBL ELISA, 82 were positive by Euroimmun ELISA, 77 reacted with recombinant human Col VII by immunoblot and 67 sera reacted with the dermal side of SSS. By MBL ELISA, only 1 BP serum was positive, while 1 BP serum and 1 PV serum were positive by Euroimmun ELISA. None of the control sera reacted with the dermal side of SSS, while 6 BP, 4 PV and 3 control sera were positive by immunoblot with recombinant Col VII. Thus, the sensitivities of the studied serological assays were: MBL ELISA: 90.8% (95% CI: 83.3–95.7%), Euroimmun ELISA: 83.7% (95% CI: 74.8–90.4%), SSS: 69.1% (95% CI: 58.9% - 78.1%), immunoblot: 79.4% (95% CI: 70.0–86.9%). The specificities of the four tests with respect to the BP and PV sera were: MBL ELISA: 99.3% (95% CI: 96.3–100.0%), Euroimmun ELISA: 98.7% (95% CI: 95.2–99.8%), SSS: 100% (95% CI: 96.3–100.0%), immunoblot: 93.2% (95% CI: 87.9–96.7%). In summary, the MBL ELISA showed significantly higher sensitivity in our samples for the detection of anti-Col VII IgG than the Euroimmun ELISA ($P = 0.008$), while specificity did not differ significantly ($P = 0.564$). Both diagnostic assays showed higher sensitivity compared to indirect IF using SSS and immunoblot with recombinant Col VII to establish the diagnosis of EBA. However, in the case of the Euroimmun ELISA and immunoblot, the difference was not significant (95% CI: −4.3% to 12.9%).

P057

The lipophilic Echinacea purpurea root extract exhibit anti-inflammatory and anti-pruritic properties *in vitro* and *in vivo*

M. Soeberdt[1], A. Olah[2], U. Knie[1], T. Biro[2], S. Dähnhardt-Pfeiffer[3] and C. Abels[1] *[1]Dr. August Wolff GmbH & Co. KG Arzneimittel, 33611 Bielefeld; [2]University of Debrecen, Debrecen, Hungary; [3]Microscopy Services Dähnhardt GmbH, Flintbek, Germany*

Echinacea purpurea extracts (purple coneflower) are known to have immunemodulatory effects. Several alkamides, as the major lipophilic constituents, bind to cannabinoid receptors 1 and 2. Since the endocannabinoid system is of importance in inflammatory skin diseases, anti-inflammatory activity of alkamides was investigated. Therefore the new lipophilic root extract of Echinacea purpurea was tested in cultured human keratinocytes. A significant ($P < 0.05$) reduction of lipoteichoic acid-induced mRNA expression of IL-1α, IL-1β, and IL-6 was observed. Moreover, the mixture was also able to reduce expression of IL-8 and showed significant anti-inflammatory effects *in vitro*.
The overall impact of the new developed water-in-oil (W/O) emulsion containing the new lipophilic root extract of Echinacea purpurea was investigated in different clinical studies.
Long-term efficacy and safety of the Echinacea purpurea root extract (W/O) emulsion was evaluated in a 3 month half-side trial against comparator (30.2 ± 15.9 years; $n = 60$). The emulsions were applied at least twice daily on two comparable and contralateral located skin areas on the crooks of arms, hollow of the knees, on the trunk, on the wrist or on the shin with slight lesions of atopic dermatitis.
Erythema reduced significantly after 1, 2 and 3 months after application of Echinacea purpurea root extract (W/O) emulsion, as well as comparator. Interestingly, Echinacea purpurea root extract (W/O) emulsion is superior after prolonged application, indicated by a significant difference to comparator after 2 and 3 months. A significant reduction of pruritus could be measured after 1, 2 and 3 months. Furthermore Echinacea purpurea root extract (W/O) emulsion was significant superior compared to comparator after 2 months and nearly significant after 3 months. The same was observed for the local SCORAD. A significant reduction could be measured after 1, 2 and 3 months. Furthermore Echinacea purpurea root extract (W/O) emulsion was significant superior compared to comparator after 3 months.
To gain further insight in underlying mechanisms, electron microscope analyses of the skin barrier as well as the lipid analysis by HPTLC will be performed and data will be presented.
In summary, application of an Echinacea purpurea root extract (W/O) emulsion reduced significantly the local SCORAD, erythema and pruritus without irritation, very likely by improved functions of the epidermal barrier.

P058

Melanoma cell expression of the PD-1 effector molecule, p-S6, correlates with response to PD-1 therapy in cancer patients

E. Guenova[1], S. Kleffel[2], C. Posch[2], A. Cozzio[1], R. Dummer[1], T. S. Kupper[2], T. Schatton[2] and W. Hötzenecker[1] *[1]Department of Dermatology, University Hospital Zurich, 8800 Zurich, Switzerland; [2]Harvard Skin Disease Research Center, Boston, USA*

Therapeutic antibodies targeting programmed cell death-1 (PD-1) activate tumorspecific immunity and have shown remarkable efficacy in the treatment of melanoma. Yet, little is known about tumor cell-intrinsic PD-1 pathway effects. We previously showed that human melanomas contain PD-1-expressing cancer subpopulations and demonstrated that melanoma cell-intrinsic PD-1 promotes tumorigenesis, even in mice lacking adaptive immunity.
To further assess the translational relevance of melanoma cell-intrinsic PD-1 receptor signaling, we quantitatively assessed melanoma-p-S6 positivity (a PD-1 effector molecule) in pre-treatment versus post-treatment tumor biopsies ($n = 11$) undergoing anti- PD-1 therapy. Additionally, in a cohort of $n = 34$ melanoma patients pre-treatment tumor tissue was stained for p-S6 expression and correlated with progression-free survival and overall survival.
We found that melanoma biospecimens sampled post PD-1 therapy demonstrated significantly ($P = 0.005$) decreased p-S6 expression compared to patient-matched pre-treatment biopsies, consistent with our findings in PD-1 antibody-treated murine and human melanoma cell lines. Additionally, in a cohort of 34 melanoma patients where pre-treatment tumor tissue was available for analysis, we found that patients with high p-S6 expression (>25% of melanoma cells) prior to treatment showed a > 3–fold increase in progression-free survival (mean progression-free survival: 17.0 vs. 4.5 months, $P = 0.001$) and significantly ($P < 0.05$) enhanced overall survival (mean overall survival: 25.1 vs. 13.0 months) compared to melanoma patients with low p-S6 levels (<25% of melanoma cells) in pre-treatment tumor biospecimens. Our findings identify p-S6 as a potential biomarker for predicting and monitoring response to PD-1 pathway blockade, thereby highlighting the possible translational relevance of melanoma cell-intrinsic PD-1 receptor functions.

P059

Diagnostic relevance of anti-desmocollin autoantibodies in pemphigus

I. Karl[1,2], S. Mindorf[1,3], I. M. Dettmann[1,3], N. van Beek[4], K. Rentzsch[3], B. Teegen[3], C. Probst[3], L. Komorowski[3], M. Kasperkiewicz[2], K. Fechner[3], W. Schlumberger[3], D. Zillikens[2,4], W. Stöcker[3] and E. Schmidt[2,4] *[1]Authors contributed equally; [2]University of Luebeck, Luebeck Institute of Experimental Dermatology (LIED), 23538 Luebeck, Germany; [3]Euroimmun AG, Institute of Experimental Immunology, 23560 Luebeck, Germany; [4]University of Luebeck, Department of Dermatology, 23538 Luebeck, Germany*

Pemphigus is a potentially life-threatening autoimmune blistering disease (AIBD) characterized by autoantibodies against desmosomal proteins. While autoantibodies in pemphigus vulgaris (PV) and pemphigus foliaceus (PF) mainly target desmoglein (Dsg) 3 and Dsg1, reactivity against non-desmoglein proteins, e.g. plakoglobin and desmocollins (Dsc), have also been reported. The autoantibody response in paraneoplastic pemphigus (PNP) is more heterogenous and Dsg3, envoplakin, periplakin, desmoplakin I / II, plectin, BP230, α2 macroglobulin-like 1, and Dsc have been described as target antigens. The pathogenic relevance of anti-Dsc IgG has previously suggested by *in vitro* studies using cultured keratinocytes. In the present study, the ectodomains of Dsc1, Dsc2, and Dsc3, respectively, were expressed on the cell surface of HEK293 cells. Dsc-expressing and control cells were applied as substrates in biochip mosaics in a multivariant indirect IF test. Three cohorts of well characterized pemphigus sera were probed for both IgG and IgA anti-Dsc reactivity, (i) classical PV patients with Dsg3 reactivity ($n = 102$), (ii) PV patients with positive direct IF microscopy but no IgG autoantibodies to Dsg3 ($n = 24$), and (iii) PNP sera ($n = 23$). In addition, 35 non-AIBD control sera were assayed. No anti-Dsc antibodies were found in the sera from classical PV patients. One PV patient serum with no Dsg-reactivity revealed anti-Dsc3 IgG and 3 PNP sera contained IgG autoantibodies against Dsc3 ($n = 2$) and Dsc2 ($n = 1$). In sera from controls, no Dsc-specific antibodies were found. In summary, anti-Dsc antibodies are found only occasionally in PNP and atypical pemphigus.

P060

Resistance to antibody-dependent cellular cytotoxicity impairs antitumor activity of Rituximab in a CD20+ mycosis fungoides

Y. Chang, M. T. Ziegler, D. Ignatova, K. Kerl, L. E. French, A. Cozzio and E. Guenova *University Hospital Zurich, Department of Dermatology, 8091 Zurich, Switzerland*

Anti-CD20 antibodies are well established in the treatment of CD20 positive B cell malignancies, antibody dependent cellular cytotoxicity being a major mode of action. Based on these observations, we initiated an anti-CD20 targeted systemic therapy with Rituximab in a highly aggressive, resistant to treatment CD4-CD20+ tumor stage mycosis fungoides. A standard therapeutic regiment, as established

for the treatment of B cell lymphoma was chosen, and a total of 4 treatment cycles were initially planned. However, against our expectations, no clinical response could be observed, but rather further progression of the mycosis fungoides with enlargement of the preexisting and development of new mycosis fungoides lesions. Reevaluation after the third cycle Rituximab revealed complete loss of the aberrant CD20 expression on the malignant T cells, and the treatment was discontinued. Moreover, laboratory analysis showed impaired antibody-dependent cellular cytotoxicity not only in the rituximab-treated CD20+ mycosis fungoides, but in patients with advanced leukemic CTCL disease in general. After a short follow-up period and additional treatment with liposomal doxorubicin, the patient deceased. Our result suggests that CD20 has no therapeutic implication when aberrantly expressed by neoplastic T cell in mycosis fungoides.

P061

Bedside assessment of intravital multiphoton tomography

V. Huck[1], C. Mess[1,2], K. Zens[1], T. R. Unnerstall[2], D. Metze[2], S. Ständer[2], T. A. Luger[2] and S. W. Schneider[1] [1]*Heidelberg University, Medical Faculty Mannheim, Experimental Dermatology, 68167 Mannheim, Germany;* [2]*University of Muenster, Department of Dermatology, 48149 Muenster, Germany*

Multiphoton tomography (MPT) enables the generation of non-invasive optical biopsies, i.e. high-resolution *in vivo* examination of human skin. By means of multiphoton excitation, several endogenous biomolecules like NADH, melanin, collagen or elastin show autofluorescence or second harmonic generation. Thus, these molecules provide information about the subcellular morphology, epidermal architecture and physiological condition of the skin and can indicate changes in cell metabolism, partly prior to clinical manifestation. Additional parameters like fluorescence decay times, measured and calculated by fluorescence lifetime imaging (FLIM) could be used for objective diagnosis by morphological and functional characterisation of the observed skin areas.

Against this background, we applied MPT-FLIM in patients suffering from inflammatory skin diseases, pruritic skin and chronic wounds in first multicentre clinical trials. Conducted in conformity to the Declaration of Helsinki and to The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guidelines, the studies were approved by the German Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM) and the local Ethics Committees of the involved medical faculties. The multiphoton tomographic set of high-resolution autofluorescence images with a penetration depth of 150 μm and fluorescence lifetime colour-coded images were compared at each time to the skin of intraindividual control areas as well as agecorrelated healthy subjects. Utilisation of automated image processing and databaseassisted analysis allowed to cope with the resulting vast amount of individual image data and correlated clinical findings. The feasibility of primary *in vivo* tracking of applied therapeutic agents further broadened our scope: On the one hand, we examined the permeation and subsequent distribution of agents directly visualised in patients' skin in short-term repetitive measurements. On the other hand, we performed MPT follow-up investigations in the long-term course of therapy.

Therefore, MPT equipped with a FLIM module as a novel clinical tool for bedside assessment may offer new insights into the pathophysiology and the individual etiopathology of skin diseases.

P062

Metabolomic profiling of psoriasis patients before and after systemic treatment

E. Rodrguez[1], H. Baurecht[1], U. Mrowietz[1], S. Gerdes[1], K. Eyerich[2], A. Todorova[2], K. Ghoreschi[3], A. Peters[4] and S. Weidinger[1] [1]*University Hospital Schleswig-Holstein, Campus Kiel, Department of Dermatology, Allergology and Venereology, Kiel;* [2]*Technische Universität München, Department of Dermatology and Allergy Biederstein, Munich;* [3]*Eberhard Karls University of Tuebingen, Department of Dermatology, Tuebingen;* [4]*Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Institute of Epidemiology II, Neuherberg*

Psoriasis is a chronic immune-mediated inflammatory skin disorder, and associated with cardiometabolic diseases. Commonly used systemic treatments are based on unspecific or targeted immunomodulation. In order to search for potential metabolic differences associated with psoriasis and to investigate the influence of systemic treatment, we conducted a prospective 12-week open-label trial on 54 adults with moderate-to-severe psoriasis treated with fumaric acid and TNFα-inhibitors. Targeted measurement of 179 metabolite serum levels was conducted with the AbsoluteIDQ p180 Kit (Biocrates Life Science AG) by mass spectrometry (MS/MS). Metabolic profiles of patients were compared to 77 age and sex matched healthy individuals from the population-based KORA study and longitudinally analysed.

Preliminary analysis revealed various metabolites of different classes to be significantly increased in untreated psoriasis patients as compared to healthy individuals ($P < 0.003$). Specifically, a significant psoriasis associated increase of different amino acid including glutamate, glycine, serine, and phenylalanine could be identified. Serum levels of these amino acids strongly decreased after treatment with systemic TNFα antagonists and showed a direct correlation with an improved post-treatment PASI score. Comprehensive subgroup analyses are currently being performed.

These data indicate that metabolomics has the potential to infer disease and treatment related biomarkers.

P063

Measurement properties of adult quality of life measurement instruments for eczema: a systematic review

D. Heinl[1], C. A. Prinsen[2], S. Deckert[2], J. Chalmers[3], A. Drucker[4], R. Ofenloch[5], R. Humphreys[6], T. Sach[7], S. L. Chamlin[8], J. Schmitt[9] and C. Apfelbacher[1,10] [1]*University of Regensburg, Regensburg;* [2]*EMGO+ Institute for Health and Care Research, Amsterdam;* [3]*University of Nottingham, Nottingham;* [4]*University Health Network, Toronto;* [5]*University Hospital Heidelberg, Heidelberg;* [6]*Budleigh Salterton, Devon;* [7]*University of East Anglia, Norwich;* [8]*Northwestern University Feinberg School of Medicine, Chicago;* [9]*TU Dresden, Dresden;* [10]*Brighton and Sussex Medical School, Brighton*

Background: The Harmonising Outcomes Measures for Eczema (HOME) initiative has identified quality of life (QoL) as a core outcome domain to be evaluated in every eczema trial. It is unclear which of the existing QoL instruments is most appropriate for this domain. Thus, the aim of this review was to systematically assess the measurement properties of existing measurement instruments developed and/or validated for the measurement of QoL in adult eczema.

Methods: We conducted a systematic literature search in PubMed and Embase identifying studies on measurement properties of adult eczema QoL instruments. For all eligible studies, we assessed the adequacy of the measurement properties and the methodological quality of the respective study with the COnsensus-based Standards for the selection of health status Measurement INstruments (COSMIN) checklist. A best evidence synthesis summarizing findings from different studies formed the basis to assign four degrees of recommendation (A-D).

Results: 15 articles reporting on 17 instruments were included. No instrument fulfilled the criteria for category A. Six instruments were placed in category B, meaning that they have the potential to be recommended depending on the results of further validation studies. Three instruments had poor adequacy in at least one required adequacy criterion and were therefore put in category C. The remaining eight instruments were minimally validated and were thus placed in category D.

Conclusions: Currently, no QoL instrument can be recommended for use in adult eczema. The Quality of Life Index for Atopic Dermatitis (QoLIAD) and the Dermatology Life Quality Index (DLQI) are recommended for further validation research.

P064

Cochrane Skin Group – Core Outcome Set Initiative (CSG-COUSIN)

L. Johannsen[1], S. Deckert[1], J. Schmitt[1], C. Apfelbacher[2] and H. Williams[3] [1]*Medizinische Fakultät Carl Gustav Carus, TU Dresden, Centre for Evidence-Based Healthcare, Dresden, Germany;* [2]*University of Regensburg, Medical Sociology, Institute of Epidemiology and Preventive Medicine, Regensburg, Germany;* [3]*University of Nottingham, Centre of Evidence-Based Dermatology, Nottingham, UK*

The choice of outcomes and adequate outcome measurement instruments in clinical trials is essential to make trial results meaningful. The failure to assess the outcomes that are most important to patients and the continued use of different outcome measurement instruments with unclear validity and reliability are frequent and constitute important barriers towards evidence-based medicine. Core outcome sets (COS) are currently developed in different medical fields to standardise and improve the selection of outcomes and outcome measurement instruments in clinical trials, in order to pool results of trials or to allow indirect comparison between interventions. A COS is an agreed minimum set of outcomes that should be measured and reported in all clinical trials of a specific disease or trial population. Outcomes additional to the COS can and should be measured as required for the specific research question.

In the field of dermatology Jochen Schmitt and Hywel Williams initiated the international, multidisciplinary Cochrane Skin Group Core Outcome Set Initiative (CSG-COUSIN) in 2014. The inaugural meeting of CSG-COUSIN became the theme of the annual CSG meeting in Dresden in March 2015. With energy and enthusiasm the international community agreed to collaboratively work on the aim on development, quality assurance, implementation, and dissemination of core outcome sets in dermatology.

Currently, CSG-COUSIN is not externally funded and relies on the enthusiasm of those individuals working within this group. An organisational structure of CSGCOUSIN has been developed: the initiative consists of the management team, the methods group and the different project groups. The management team coordinates CSG-COUSIN and provides technical and organizational support for the methods group and project groups, e.g. with an information management. The methods group provides methodological support and internal peer review for CSG-COUSIN project groups, aims to conduct methodological studies on outcomes research and COS development, and sets up quality standards for COS development and implementation processes. CSG-COUSIN project groups work on the development and implementation of specific COS in dermatology. Project groups consist of a lead, patient representative, member of the methods group, and other group members representing different stakeholder groups and geographical regions. The project group for eczema – the Harmonising Outcome Measures for Eczema (HOME) initiative – already set out to develop a COS for eczema trials in 2010. HOME also suggested a roadmap to guide the process of core outcome and core outcome measurement selection and implementation. Other COS initiatives on hidradenitis suppurativa, hand eczema, vascular malformations, and urticaria have been initiated within CSG-COUSIN or have affiliated with CSG-COUSIN. CSG-COUSIN is open for every interested person (COUSIN@uniklinikum-dresden.de).

P065

6- and 8-prenylnaringenin have direct anticancer properties, activate natural killer (NK) cells and improve NK cell-mediated killing of cancer cells

C. Busch[1,2], M. Burkard[1], C. Leischner[3] and S. Venturelli[3] [1]*University of Tuebingen, Dermatology, Tuebingen, Germany;* [2]*Pallas-Klinik, Winterthur, Switzerland;* [3]*University of Tuebingen, Internal Medicine I, Tuebingen, Germany*

Flavonoids form an essential group of secondary plant metabolites, which gained increasing attention due to a broad range of promising health effects described *in vitro* and *in vivo*. The medicinal plant Humulus lupulus (hops) contains a large amount of flavonoid derivatives, particularly prenylated flavonoids such as 6- prenylnaringenin (6-PN) and 8-prenylnaringenin (8-PN). Compared to 'classical flavonoids' only little is known about the biological activity of these prenylated flavonoids.

We previously described a novel HDAC-inhibitory activity of 6-PN and 8-PN. Considering the globally increasing cancer incidence, there is urgent need for novel drugs.

An ideal anticancer drug should (i) exert anticancer activity, (ii) be well-tolerated by non-malignant tissues, and (iii) not impair the immune system.

i Here we show potent antiproliferative activity of 6-PN and 8-PN at 6.25–50 μM towards various human cancer cells (prostate, renal, liver, melanoma, lung, breast).

ii Both 6-PN and 8-PN were well-tolerated at similar concentrations by benign human cells and tissues (colon cells, skeletal muscle cells, primary hepatocytes, bone marrow, PBMCs).

iii In contrast to the clinically approved HDAC-inhibitor vorinostat (SAHA), which dramatically decreased NK cell viability at 1–10 μM, both 6-PN and 8-PN increased NK cell viability at 6.25–50 μM, independent of IL-2. Further, 6-PN and 8-PN at 6.25–50 μM increased long-term survival of NK cells. Finally, 6-PN and 8-PN at 25 μM synergistically increased NK cell-mediated killing of human hepatocellular cancer cells at an effector to target ratio of 1.25:1.

Due to these results 6-PN and 8-PN are currently evaluated in a clinical phase I trial for bioavailability and bioactivity in healthy volunteers.

P066

A novel preclinical model of organotypic slice cultures for pharmacodynamic profiling of human melanomas

H. Niessner[1], T. Eigentler[1], P. Bauer[2], M. Levesque[3], C. Garbe[1] and T. Sinnberg[1] [1]*University of Tuebingen, Department of Dermatology, 72076 Tuebingen, Germany;* [2]*University of Tuebingen, Insitute of Medical Genetics and Applied Genomics, 72076 Tuebingen, Germany;* [3]*University of Zuerich Hospital, Department of Dermatology, CH-8091 Zurich, Swizerland*

Predicting drug response in melanoma patients remains a major challenge in the clinic. We have established an ex vivo, reproducible, rapid and personalized culture method to investigate anti-tumoral and pharmacological properties. The response to signal transduction inhibitors and therapy efficacy is determined not only by properties of the drug target but also by concomitant mutations in other signaling molecules and the tumor microenvironment. Therefore, a solid and fast functional test system that preserves melanoma microenvironment and tumor heterogeneity is of great interest.

Melanoma punch biopsies or patient derived xenograft tumors were used for the preparation of 400 μm thin tissue slices using a vibratome. The slices were cultivated for five days and treated for four days with clinical relevant drugs like BRAF or MEK inhibitors before measuring tissue viability by an enzymatic assay. Tissue slices were further used for immunohistochemical evaluation of proliferation (Ki67) and apoptosis induction (cleaved PARP). The results were correlated to the genetic background of the tumor and the clinical data of the patient.

Our results show that this slice culture model preserves tissue 3D architecture, cell viability and pathway activity up to 5 days ex vivo. Treatment of melanoma slice cultures with inhibitors reduced tissue viability in a reproducible manner and correlated to the clinical efficiency and known resistant mechanisms. Effects of the drugs on tumor cell proliferation and apoptosis were successfully determined by Ki67 and cleaved PARP stainings.

P067

Hunt for somatic mutations in Linear Localized Scleroderma

R. Higgins[1,2], R. Wälchli[1,2], A. Smith[1,2], M. Theiler[1,2], L. Weibel[1,2] and A. Navarini[1] [1]*Universitäatspital Zürich, Department of Dermatology, 8052 Zurich, Switzerland;* [2]*Kinderspital Zurich, Department of Dermatology, 8052 Zurich, Switzerland*

Linear localized scleroderma (LLS) is a rare connective tissue disorder characterised 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 including arms, legs and more rarely the face in the clinical subtypes en coup de sabre and Parry-Romberg syndrome. This leads to terrible disfigurement. The disease affects mostly children and is limited in treatment options which are most often unsatisfactory. The incidence of localized scleroderma in adults and children is 2.7/100 000 population per year.

As of yet very little is understood about the condition in terms of both genetic and clinical aetiology. There is increasing evidence that that LLS might be based on genetic alterations in affected tissues. In dermatology, multiple skin conditions have been shown to follow Blaschko's lines, the patterns of cell migration during embryological development. Several of these diseases have been demonstrated to be caused by genetic factors such as a de novo somatic mutation causing a cutaneous mosaicism. The aim of this project is to describe the genetic architecture of LLS in the hope that this could lead to a better understanding of the disease through whole exome sequencing to find candidate genes.

P068

Wundproteom als holistischer Ansatz für Diagnostik und Therapie chronischer Wunden

G. Daeschlein[1], C. Sicher[2], G. Wachlin[2], M. Jünger[1] and K. Riedel[2] [1]*Universitätsmedizin Greifswald, Dermatologie, 17475 Greifswald, Deutschland;* [2]*Institut für Mikrobiologie, Mikrobiologie Proteomics, 17487 Greifswald, Germany*

Chronische Ulcuswunden entstehen durch hämodynamische Störungen verschiedener Genese, die letzten Endes zu einem Energiedefizit im Gewebe und konsekutiv zu Nekrosen führen. Die Entzündungsreaktion führt u.a. zu einem Anstieg verschiedenster Proteasen, die in Gegenwart pathologisch veränderter Regulatoren unphysiologisch erhöht bleiben und über permanente degradierende Aktivität mit Abbau der extrazellulären Matrix eine adäquate Wundheilung besonders in der Remodeling- und Reepithelisierungsphase unmöglich machen. Bisherige Studien konnten im Sekret chronischer Wunden verschiedenste hochregulierte Enzyme nachweisen, wobei Metalloproteinasen wie die Gelatinase A (Metalloproteinase 2, MMP 2) und Gelatinase B (MMP 9) sowie verschiedene Serinproteasen wie Kathepsin G und neutrophile Elastase eine zentrale Rolle spielen. Bei chronischen Wunden konnten erhöhte Konzentrationen von MMP 2, 8 und 9, außerdem der humanen neutrophilen Elastase nachgewiesen werden. Insgesamt liegen bisher z.T. uneinheitliche Ergebnisse vor, die zum Teil auf die unterschiedlichen Nachweisverfahren zurückzuführen sind. Daher untersuchten wir die regulatorischen Proteine im Wundsekret mit einem holistischen Ansatz, der über den gleichzeitigen Nachweis aller beteiligten Reaktanden mittels Proteom-Analyse für die Klärung der offene Fragen aussichtsreich erscheint.

Methoden: Aus Wundabstrichen von 8 Patienten mit unterschiedlichen Wunden wurde nach Proteinfällung ein Proteom erstellt und massenspektrometrisch analysiert, die Proteine anhand von webbasierten Datenbanken zugeordnet. Nach Normalisierung wurden die counts der einzelnen Proteine auf Signifikanz geprüft.

Ergebnis: Bei allen untersuchten Sekreten konnten im Proteom 300–500 Proteine nachgewiesen werden. In den Proben wurden diverse Proteine der einschlägigen regulativen Proteinfamilien detektiert. Zu den Proteinfamilien, bei denen mehrere Proteine bei allen Sekreten auftraten gehörten Heat Shock-Proteine, Zytoskelettproteine, Extrazelluläre Matrixproteine, Immunglobuline und Complementproteine, Proteine des Kohlenhydrat- und Lipidstoffwechsels, Metalloproteinasen, Peptidasen und Proteaseinhibitoren, Reaktive oxidative Spezies (ROS), Signaltransduktion und Transportproteine.

Signifikant vermehrt zeigte sich MMP8 und MMP9 bei chronisch venöser Insuffizienz nur bei langjähriger Ulcusanamnese, S100 A9 bei gemischtem Ulcus und Myeloblastin bei tumorbedingter Heilungsstörung. Flache Ulcera mit Papillomatosen zeigen keine signifikanten Proteinveränderungen.

Schlußfolgerung: Wundproteomics als sensitive und zuverlässige Methode zeigt Momentaufnahmen der verschiedenen regulativen Heilungsprozesse in der Wunde mit vergleichbaren Aktivitätsbewertungen und erlaubt somit Hinweise auf die Ätiologie chronischer Wunden. MMP 8 und 9 scheinen pathognomonisch für nicht heilende CVI-Wunden. Dies erscheint für diagnostische und auch therapeutische Ansätze aussichtsreich.

Dermato-Endocrinology

P069

Calcipotriol treatment increases low levels of cathelicidin expression and enhances anti-microbial activity of recessive dystrophic epidermolysis bullosa keratinocytes

C. Gruber[1], J. Pión Hofbauer[1], B. Tockner[1], C. Hüttner[1], J. S. Breitenbach[1], A. Trost[2], V. M. Leb-Reichl[1], A. Klausegger[1], S. Hainzl[1], J. Reichelt[1], J. W. Bauer[3] and R. Lang[3] [1]*EB House Austria, Research Program for Molecular Therapy of Genodermatoses, Department of Dermatology, University Hospital of the Paracelsus Medical University, Salzburg, Austria, 5020 Salzburg, Austria;* [2]*University Clinic of Ophthalmology and Optometry, Research program for Ophthalmology and Glaucoma Research, Paracelsus Medical University Salzburg, Austria, 5020 Salzburg, Austria;* [3]*Department of Dermatology, University Hospital Salzburg, Paracelsus Medical University Salzburg, Austria, 5020 Salzburg, Austria*

Recessive dystrophic epidermolysis bullosa (RDEB) is a severe genetic skin blistering disease caused by the absence of anchoring fibrils that function to attach the epidermis to the underlying dermis. Consequently, RDEB patients suffer from persistent wounds predisposing them to microbial infections which contribute to delayed wound healing and ongoing inflammation, both of which promote the development of an aggressive squamous cell carcinoma in these patients. Thus, local wound care and antimicrobial defense is critical in EB wound management.

Antimicrobial peptides (AMPs) form part of the body's innate immune response and serve as potent antibiotics that control pathogenic infections and activate the adaptive immune system. One of the most prominent AMP in human epithelial cells is cathelicidin (also known as hCAP18) which is not only capable of augmenting host defense, but also appears to play a role in tissue repair and wound closure. We observed reduced levels of hCAP18 expression in keratinocytes and skin tissue samples from RDEB patients which may in part contribute to the increased susceptibility of patients to infection. Notably, we were able to upregulate hCAP18 expression in immortalized RDEB keratinocytes using the vitamin D analogue calcipotriol, which has previously been shown to be a potent activator of cathelicidin transcription in human keratinocytes. Furthermore, calcipotriol treatment of RDEB keratinocytes resulted in induction of anti-microbial activity against Escherichia coli as demonstrated by reduced colony formation upon incubation with supernatants from calcipotriol-treated RDEB cells. The observed bactericidal activity correlated with a robust induction of hCAP18 in these cells, suggesting that these effects were mediated by cathelicidin. Although Vitamin D is known to inhibit proliferation of human keratinocytes, we observed no anti-proliferative effect on RDEB cell lines except at the highest concentration (1000 nM) investigated, thus pointing to its applicability in wound healing studies in RDEB.

In summary our data highlight cathelicidin as a potential therapeutic target to enhance antimicrobial defense and improve wound healing in RDEB. Currently we are investigating the effect of this treatment strategy towards more skin relevant pathogens such as Pseudomonas aeruginosa, as well as characterizing potential defects in the vitamin D – cathelicidin pathway in RDEB which may also affect local response of keratinocytes to wounding stimuli in this patient group.

P070

Tropisetron modulates the UVA response in human dermal fibroblasts – a novel function of the α7 nicotinic acetylcholine receptor

A. Stegemann, M. Apel and M. Boehm *University of Muenster, Department of Dermatology, 48149 Muenster, Germany*

Cutaneous photoaging is crucially mediated by ultraviolet A (UVA) irradiation. Upon UVA exposure reactive oxygen species (ROS) including hydrogen peroxide (H2O2) are generated in fibroblasts resulting in elevated expression of matrix metalloproteases (MMPs) and thus in tissue degradation. Here, we hypothesized that tropisetron, an approved antiemetic substance originally characterized as a serotonin receptor modulating agent, may exhibit anti-oxidative effects in human dermal fibroblasts (HDFs) exposed to UVA. Previously we showed that this agent has antifibrotic effects in the mouse model of scleroderma and reduces collagen synthesis in HDFs via an off-target effect, i. e. activation of α7 nicotinic acetylcholine receptors (α7nAchRs). To test our hypothesis we pretreated HDFs with tropisetron and irradiated them with UVA followed by detection of ROS. Pretreatment of HDFs for 24 h with tropisetron led to significantly reduced intracellular amounts of ROS as well as extracellular levels of H2O2 upon UVA exposure. Moreover, we found a significant suppression of UVA-induced MMP1 and MMP3 mRNA expression in HDFs as determined by real-time RT-PCR. Receptor analysis of the putative tropisetron receptors disclosed that the serotonin receptors 5-HT3-R and 5-HT4- R are not detectable in HDFs. In contrast, we found an expression of the α7nAchR in HDFs as shown at RNA level by semi-quantitative RT-PCR as well as at protein level by immunofluorescence analysis. In support of these findings, AR-R17779, a full agonist of the α7nAchR reduced UVA-induced generation of H2O2 in HDFs. Treatment of HDFs with catalase diminished UVA-induced H2O2 accumulation suggesting that tropisetron elicits its anti-oxidative effect via activating the antioxidative enzyme catalase. In summary, our findings have identified a novel antioxidative lead substance that acts via α7nAchRs and which suppresses cellular responses of fibroblasts exposed to UVA irradiation.

P071

A chemically modified derivative of the anti-inflammatory tripeptide KdPT (WOL074-009) ameliorates ongoing psoriasis and colitis

J. Koch[1], M. Steinert[1], S. Koch[1], M. Soeberdt[2], U. Knie[2], C. Abels[2], T. A. Luger[1] and K. Loser[1] [1]*University of Muenster, Department of Dermatology, 48149 Muenster, Germany;* [2]*Dr. August Wolff Gmbh & Co. KG – Arzneimittel, 33611 Bielefeld*

KdPT, a tripeptide closely related to the C-terminal amino acids of alpha-melanocytestimulating hormone (α-MSH) exhibits anti-inflammatory and immunomodulatory effects, which are predominantly mediated by the reduction of nuclear factor κB (NF κB) activation and translocation. Previously, we have shown that KdPT ameliorated ongoing imiquimod-induced psoriasis-like skin inflammation in mice by inducing tolerogenic dendritic cells and expanding regulatory T cells (Treg). However, due to its unfavourable PhysChem properties KdPT is not suitable for topical application. Hence, we chemically modified the tripeptide at the N-terminal end and by alkylation of the D-Pro-Thr amide bond resulting in the KdPT derivative WOL074-009 to improve its PhysChem properties and thus, its ability to penetrate the skin barrier. To investigate the anti-inflammatory and immunomodulatory potential of WOL074-009 a psoriasis-like skin inflammation was induced in mice by topical application of imiquimod for 8 consecutive days. At day 4 and 6 after the start of imiquimodtreatment, when skin inflammation had established, mice were intravenously injected with PBS, KdPT or WOL074-009 (5 μg per mouse and day). Interestingly, similar to KdPT, WOL074-009 treatment ameliorated ongoing skin inflammation as shown by the reduced epidermal thickness, decreased elongation of epidermal rete ridges and the down-regulated levels of pathogenic T cells in regional lymph nodes and lesional skin. This effect was mediated by the reduction of pro-inflammatory cytokines like IL-1β, IL-6 or TNF-α and the expansion of immunosuppressive Treg in WOL074-009-treated mice compared to PBS-treated controls. To investigate whether WOL074-009 was able to ameliorate inflammation in other epithelial tissues than the skin we induced colitis in mice by adding 2.5% dextrane sodium sulphate (DSS) to the drinking water resulting in severe weight loss and the induction of rectal bleeding in PBS-injected control animals within 8 days. Interestingly, mice that were intraperitoneally injected with 25 μg WOL074-009 at day 4–8 after the start of DSS treatment were protected from weight loss and moreover, did not show any signs of diarrhoea. Additionally, quantitative real-time PCR as well as immunofluorescence staining revealed decreased levels of pro-inflammatory cytokines and reduced numbers of neutrophils or macrophages in mesenteric lymph nodes as well as the colon from WOL074-009-treated mice versus PBS-injected controls pointing to a potent anti-inflammatory effect of WOL074-009 in DSS-induced colitis. Together, WOL074-009, similar to the original tripeptide KdPT, is able to ameliorate ongoing inflammation in epithelial barrier tissues of the skin and the gut. Because of the improved PhysChem properties of WOL074-009 as compared to KdPT and due to the possibility of topical application, our data might suggest WOL074-009 as a potential therapeutic option for the treatment of patients with inflammatory skin diseases.

P072

A new LC-MS/MS assay for the analysis of sulfated steroids in human serum: quantification of cholesterol sulfate for the diagnosis of recessive X-linked Ichthyosis

A. Sánchez-Guijo[1], V. Oji[2], M. F. Hartmann[1], H. Traupe[2] and S. A. Wudy[1] [1]*Justus-Liebig-University, Centre of Child and Adolescent Medicine, Steroid Research & Mass Spectrometry Unit, 35392 Giessen, Germany;* [2]*University of Muenster, Department of Dermatology, 48149 Muenster, Germany*

Recessive X-linked ichthyosis (RXLI) is the typical skin phenotype caused by steroid sulfatase deficiency. When compared with healthy males, the only sulfated steroids elevated in the serum of RXLI patients, with independence of their age, are cholesterol sulfate (CS) and oxysterol sulfates (hydroxylated forms of CS).

We developed and validated an LC-MS/MS bioassay to quantify those sulfated steroids found in higher concentrations in human serum (i.e. DHEAS, androsterone sulfate, pregnenolone sulfate or androstenediol-3-sulfate), allowing for a reliable determination of CS as well. To our knowledge, this method provides the most detailed profile of steroid sulfates to date. The parameters for CS, studied at 3 different quality control levels (QC), met the standards of FDA and EMA. Linearity for CS was good ($R2 > 0.99$), and recovery was within 100 ± 15% for all QCs. Precisions and accuracies (intra-day and between-day) were below 15% at all QCs of CS. The method requires only 300 μl of serum.

We applied the assay to quantify the levels of CS in serum from patients with ichthyoses. RXLI patients showed an increase of more than 30 times in CS when compared with patients with ichthyosis vulgaris or with healthy controls. The method provides a quick tool for the diagnosis of steroid sulfatase deficiency.

P073

Growth hormone as a new player in human hair follicle biology

M. Alam[1], D. A. Below[1], R. Clayton[2], J. Chéret[1,3], M. Bertolini[1,3] and R. Paus[2] [1]*University of Muenster, Dermatology, 48149 Muenster, Germany;* [2]*University of Manchester, Dermatology, Manchester, UK;* [3]*Monasterium Laboratory, 48149 Muenster, Germany*

Growth hormone (GH) and its receptor (GHR) promote cell growth, proliferation, differentiation and stem cell activation either directly or via the induction of IGF-1. While some clinical case reports suggest that hair follicles (HFs) may be GHresponsive and GHR immunoreactivity has been reported in the HF of some species, whether GH exerts any physiologically important functions in human HFs remains entirely unknown. To explore these functions we charted GH and GHR expression in human scalp HFs. So far, we have found GHR protein expression in the ORS of human anagen VI HFs as well as in the sebaceous gland epithelium. Preliminary evidence suggests that HFs may also transcribe GHR ligand (GH mRNA). qRT-PCR analysis also suggested that GHR transcription decreases during HF regression (catagen HFs). Interestingly, the level of GHR transcription was inversely correlated with that of GH-inhibiting hormone, somatostatin, whose expression actually increased in catagen HFs. Next, organ-cultured human scalp HFs were stimulated with recombinant hGH (rhGH, 50–100 ng/ml) or recombinant GH-binding protein (rGHBP) (GHBP, derived from the cleavage of the extracellular domain of GHR, can exert both GH-antagonistic and agonistic effects). hGH and GHBP both promoted hair shaft elongation. Hair cycle staging showed a tendency towards catagen retardation by both GH and GHBP-treatment. qRT-PCR analysis showed that hGH (100 ng/ml) and GHBP (100 ng/ml) induced an increase in levels of JAK2, which is the downstream target after GH ligand binds to GHR. Interestingly hGH also increased the levels of IGFBP3, which has been shown to be lower in patients with vertex balding. In addition levels of IGF-1 were relatively unchanged after stimulation with both hGH and GHBP. Currently we are investigating the effect of GHR antagonist and/or knock down on standard hair biology read-out parameters and are investigating whether growth hormone releasing hormone (GHRH) impacts on intrafollicular on GH and/or GHR expression.
Taken together this pilot study already suggests that GH-induced signaling operates as a novel neuroendocrine regulator of human hair growth ex vivo.

P074

Skin pigmentation, cutaneous vitamin D synthesis and evolution: variants of genes (SNPs) involved in skin pigmentation are associated with 25(OH)D serum concentration

W. Roßberg[1], J. Schöpe[2], R. Saternus[1], S. Wagenpfeil[2], M. Kleber[3], W. März[3], T. Vogt[1] and J. Reichrath[1] [1]*Saarland University, Department of Dermatology, Homburg, Germany;* [2]*Saarland University, Institute for Medical Biometry, Epidemiology and Medical Informatics, Homburg, Germany;* [3]*Ruperto-Carola University of Heidelberg, Mannheim Institute of Public Health, Heidelberg, Germany*

In Caucasian populations, vitamin D deficiency is common and associated with higher risk for and unfavourable outcome of many diseases, including various types of cancer, infectious, cardio-vascular, and autoimmune diseases. Individual factors that predispose for a person's vitamin D status, including skin type, have been identified, but limited data exist on genetic determinants of serum 25(OH)D concentration. We have tested the hypothesis that variants of genes (SNPs) involved in skin pigmentation are predictive of serum 25(OH)D levels. Serum 25(OH)D and SNPs ($n = 244$) within genes ($n = 15$) ATP7A, DTNBP1, BLOC1S5, PLDN and PMEL (involved in melanosomal biogenesis); RAB27A, MYO5A and MLPH (encoding transfer proteins relevant for the melanosomal transport within the melanocyte); MC1R, MITF, PAX3, SOX10, DKK1, RACK1 and CNR1 (involved in melanocyte signaling pathways) were analyzed in a cohort of participants of the LURIC study. We included 2974 patients (29.83% females, 70.17% males) with a mean serum 25(OH)D concentration of 17.3 ng/ml (median 15.5 ng/ml). The following 11 SNPs located in 7 different genes were associated ($P < 0.05$) with lower or higher serum 25(OH)D levels (medians from highest to lowest): rs6454677 (CNR1), 22.5 ng/ml, $P = 0.046$; rs2069408 (PMEL), 17.05 ng/ml, $P = 0.015$; rs2292881 (MLPH), 16.8 ng/ml, $P = 0.041$; rs7569427 (MLPH), 15.3 ng/ml, $P = 0.026$; rs9328451 (BLOC1S5), 14.6 ng/ml, $P = 0.028$; rs10932949 (PAX3), 13.9 ng/ml, $P = 0.004$; rs7565264 (MLPH), 13.1 ng/ml, $P = 0.000992$; rs17139617 (ATP7A), 12.85 ng/ml, $P = 0.000096$; rs2227291 (ATP7A), 12.8 ng/ml, $P = 0.000047$; rs10521358 (ATP7A), 12.8 ng/ml, $P = 0.000025$; rs12469812 (MLPH), 12.5 ng/ml, $P = 0.030$. 3 out of these 11 SNPs reached the aimed significance level after correction for multiple comparisons (FDR). In the linear regression model adjusted for sex, body mass index (BMI), year of birth and month of blood sample these SNPs showed a significant association with 25(OH)D: rs7565264 (MLPH), rs10932949 (PAX3), rs9328451 (BLOC1S5). The combined impact on the variation of 25(OH)D serum levels (coefficient of determination (R^2)) for the 11 SNPs was 1.6% and for the 3 SNPs after FDR 0.3%. We also checked if the SNPs have a significant association with the survival of the patients. In Cox Regression we identified rs2292881 (MLPH) for having a significant advantage in overall survival. Kaplan-Meier analysis did not show a significant impact of individual SNPs on overall survival. Our results have a fundamental importance to understand the role of sunlight, skin pigmentation and vitamin D for the human evolution.

P075

Vitamin D suppresses caspase-5 and IL-1beta release by epidermal keratinocytes in psoriasis

S. Zwicker[1,2], E. Hattinger[1], D. Bureik[1], A. Batycka-Baran[1,3], A. Schmidt[4], S. Rothenfusser[4], M. Gilliet[5], T. Ruzicka[1] and R. Wolf[4] [1]*Ludwig Maximilian University, Department of Dermatology and Allergology, 80337 Munich, Germany;* [2]*Karolinska Institute, Department of Dental Medicine, 141 52 Huddinge, Sweden;* [3]*Wroclaw Medical University, Department of Dermatology, Venereology and Allergy, 50-368 Wroclaw, Poland;* [4]*Ludwig Maximilian University, Division of Clinical Pharmacology, Medizinische Klinik IV, 80336 Munich, Germany;* [5]*CHUV University Hospital, Department of Dermatology, University Hospital of Lausanne, 1011 Lausanne, Switzerland*

IL-1beta is a potent player in cutaneous inflammation and important for the development of the Th17 micro-milieu in autoinflammation. Its activity is controlled on transcriptional level and by subsequent proteolytic cleavage by inflammasome complexes. Recently, the NLRP1 inflammasome has been genetically linked to Th17- mediated autoinflammatory diseases including psoriasis. We report the NLRP1- inflammasome active in human epidermal keratinocytes and increased in psoriatic skin lesions. Topical vitamin D analogues are standard treatment in psoriasis but its functional effect on epidermal IL-1beta production is unknown. Here, we showed that vitamin D interfered with the IL-1beta release and suppressed caspase-5 in keratinocytes and in psoriatic skin lesions. Thus, data uncovered NLRP1-dependent caspase-5 activity as a therapeutic targets in psoriasis and provide a novel antiinflammatory mechanism for vitamin D in Th17-mediated skin autoinflammation.

P076

Vitamin D status is associated with serum lipid profile in participants of the Ludwigshafen Risk and Cardiovascular Health (LURIC) study

A. Merkoureas[1], S. Wagenpfeil[2], M. Kleber[3], W. März[3], T. Vogt[1] and J. Reichrath[1] [1]*Saarland University, Department of Dermatology, Homburg, Germany;* [2]*Saarland University, Institute for Medical Biometry, Epidemiology and Medical Informatics, Homburg, Germany;* [3]*Ruperto-Carola University of Heidelberg, Mannheim Institute of Public Health, Heidelberg, Germany*

Vitamin D deficiency has been associated with bone diseases and many unrelated health disorders. However, little is known about the impact of vitamin D status on serum lipids. The aim of this large retrospective cohort study ($n = 3316$) was to analyze the potential association of vitamin D status with an extensive panel of serum parameters of lipid metabolism (cholesterol, HDL, LDL, triglycerides, VLDL, VLDL-triglycerides, apolipoproteins A1, A2, B, C2, C3, E) in participants of the Ludwigshafen Risk and Cardiovascular Health Study (LURIC study). Regression analysis showed a strong association of 25(OH)D and 1,25(OH)2D status with HDL, Apo A1 and Apo A2 serum concentration ($P < 0.001$). Additional statistical tests, including gender and age in multiple analyses, confirmed these findings. Subgroup analysis revealed similar results in participants with or without lipid lowering medication. Interestingly, association of vitamin D status with most serum parameters of lipid metabolism was stronger in the subgroup of participants with 25(OH)D serum concentrations <30 ng/ml, while there was no or weaker association in the subgroup of participants with higher 25(OH)D serum concentrations. Overall, the effect of vitamin D status on serum lipids was rather small (low R2 values), i.e. an increase of 1 ng/ml in 25(OH)D serum concentration resulted in an increase of 0.13 mg/dl in HDL serum concentration. In conclusion, our study supports the concept that vitamin D sufficiency exerts beneficial effects on serum lipids, reaching a plateau at 25(OH)D serum concentrations >30 ng/ml.

P077 (O03/05)

Genetic targeting of sebocytes reveals sebaceous lipids to be essential for water repulsion, thermoregulation, and the maintenance of ocular integrity in mice

M. Dahlhoff[1], D. Riethmacher[2] and M. R. Schneider[1] [1]*LMU Munich, Gene Center, 81377 Munich, Germany;* [2]*Nazarbayev University, School of Medicine, Astana, Kasachstan*

Sebocytes are epithelial cells characterized by progressive lipid accumulation leading to cell disruption. Studying the function of sebaceous gland (SG) lipids has been hampered by the lack of genetic models allowing sebocyte targeting while maintaining intact epidermal lipids. To establish a mouse line with SGspecific expression of cre recombinase, we replaced the first exon of Scd3, a gene encoding an enzyme of the Stearoyl-coenzyme A desaturase family that is expressed exclusively in sebocytes, with the cDNA for codon-improved cre recombinase via homologous recombination in embryonic stem cells. After obtaining germline transmission of the modified allele via chimeric mice, we crossed the positive offspring to the Rosa26-LacZ reporter line. Recombination of the reporter locus, examined by histochemical detection of β-galactosidase (β-gal) in animals heterozygous for both alleles, confirmed that cre activity in both back and tail skin was limited to the SG, with no staining in the epidermis, dermis, or hair follicle. As expected, β-gal staining was also evident in free SGs (Meibomian gland and preputial gland).
To assess whether depletion of differentiated sebocytes affected functions that have been attributed to sebum, we employed a diphtheria chain A toxin-mediated cell ablation approach. After swimming for 2 min in 30°C water, Scd3-cre+/wt+DTA mice looked much wetter than controls, and while control mice appeared nearly dry after 20 min, Scd3-cre+/wt+DTA mice were still wet even after 50 min. The delayed drying correlated well with a significant increase in water retention during the whole period. Both control and Scd3-cre+/wt+DTA mice had a body temperature of approximately 35.2°C before swimming. In control mice, the temperature dropped to 32.8°C immediately after swimming and returned to the normal value already 10 min later. In contrast, the temperature of Scd3-cre+/wt+DTA mice dropped to 29.6°C immediately after swimming, and remained lower than normal for ~ 30 min. Thus, loss of sebaceous lipids resulted in impaired water repulsion and thermoregulation.
Long-term observation of Scd3-cre+/wt+DTA mice revealed an eye disorder that became macroscopically visible from 3 months of age and was characterized by narrow eye fissures, eyeball opacity, frequent blinking, and signs of eye inflammation. Analysis of H&E-stained sections revealed an almost complete depletion of mature sebocytes in Meibomian glands of Scd3-cre+/wt+DTA mice, and Nile red staining confirmed a massive reduction in the lipids synthesized by the gland. Histologically, no significant alterations were observed in the cornea at 3 weeks or two months of age. In contrast, all nine examined Scd3-cre+/wt+DTA mice at 8 months of age showed chronic, severe keratoconjunctivitis sicca characterized by hyperkeratinization of the corneal epithelium accompanied by purulent inflammation of secondary origin. Thus, loss of Mebomian gland lipids caused a severe pathology of the ocular surface resembling human Meibomian gland dysfunction.
In summary, our results indicate that Scd3-cre mice can be successfully used to drive recombination specifically in sebocytes. This new mouse line will therefore permit, for the first time, assessing the specific roles of sebaceous lipids without confounding influences from the concomitant loss of epidermal lipids. These mice may also represent an important model for studying the pathogenesis and possible therapeutic strategies of Meibomian gland dysfunction in humans.

P078 (O04/05)

Oxidative stress induces proopiomelanocortin expression independently of the tumor suppressor gene product p53 in human keratinocytes

M. Boehm, M. Apel, A. Stegemann and B. Ringelkamp *Department of Dermatology, 48149 Muenster, Germany*

Proopiomelanocortin (POMC) is the precursor for melanocortin peptides and betaendorphin which are important mediators of skin tanning induced by ultraviolet (UV) light. A key role in the regulation of POMC expression in keratinocytes is attributed to the tumor suppressor gene product p53 which is well known to be induced after UVB irradiation (Cui et al., Cell 2007). We challenged this concept by hypothesizing that reactive oxygen species (ROS) – the earliest and most proximal intracellular signal transduction mediators – may also induce POMC expression. Indeed, accumulation of intracellular ROS was detectable in a dose- and time-dependent manner in normal human keratinocytes (NHKs) after UVB irradiation *in vitro*. Treatment with catalase confirmed that UVB-generated intracellular ROS in these cells represent mainly hydrogen peroxide. Hydrogen peroxide, which is found in μM doses in UVB-exposed human epidermis even *in vivo*, in fact induced POMC expression in a dose- and time-dependent fashion in NHKs *in vitro*. This effect was transcriptionally mediated. Protein expression and phosphorylation of p53 was unaffected by treatment with hydrogen peroxide. Moreover, gene knock-down or pharmacological suppression of p53 by pifithrin retained POMC induction by hydrogen peroxide in NHKs indicating that this effect is truly p53-independent. In accordance with this SaOs-2 cells lacking p53 expression likewise responded to hydrogen peroxide with increased POMC expression. In order to decipher the potential mechanism of p53-independent POMC induction by hydrogen peroxide in NHKs we focused on the nuclear orphan receptor family members NR4A1 (Nurr77) and NR4A2 (Nurr1). Both receptors are known to govern POMC expression in the pituitary gland. Interestingly, hydrogen peroxide within minutes induced dramatic expression of both NR4A1 and NR4A2. Moreover, NR4A1 rapidly migrated to the nucleus upon treatment with hydrogen peroxide. In summary, our findings highlight a novel pathway of p53-independent induction of the POMC gene in NHKs and suggest that hydrogen peroxide via regulation of nuclear orphan receptor family members is a unrecognized physiological regulator of POMC expression in the skin.

P079

Melatonin and its metabolites AFMK and AMK counteract UVR-mediated oxidative stress and functional disturbances within mitochondria in keratinocytes and fibroblasts

K. Kleszczynski, M. Stegmann, N. Kruse, D. Zillikens and T. W. Fischer *University of Luebeck, Department of Dermatology, Allergology and Venerology, 23538 Luebeck, Germany*

Melatonin (N-acetyl-5-methoxytryptamine) is an ubiquitous molecule with many different functions, including potent radical scavenging capacities. Due to its lipophilic character, it easily crosses biological membranes reaching intracellular organelles. Here, apart from melatonin (MEL), we evaluated the effect of its metabolites i.e., N1- acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK) in a dose-dependent manner (10^{-3}, 10^{-4}, 10^{-6} M) in human epidermal keratinocytes (NHEK) as well as in human dermal fibroblasts (HDF) which were exposed to UVR at the dose of 50 mJ/cm^2. First experiments using the MTT viability assay revealed that MEL or AFMK and AMK significantly protected the cells from the lethal UVR irradiation. Subsequent investigations showed a distinct UVRinduced increase of reactive oxygen species (ROS) by 34% ($P < 0.001$, NHEK) and 45% ($P < 0.001$, HDF) compared to the control. MEL, AFMK and AMK prominently counteracted oxidative stress in both cell lines, however, the most potent effect was observed in presence of AMK at the dose of 10^{-3} M. As a consequence, hypergeneration of ROS (fluorescence labeling) mediated the perturbations within the cells particularly inducing the alterations of their bioenergetics. Our data showed that UVR in the first row significantly affected the oxidative phosphorylation within mitochondria by dissipation of the mitochondrial transmembrane potential ($mt\Delta\Psi$) (fluorescence labeling). Furthermore, alterations in ATP synthesis (ELISA) were observed, and massive influx of calcium (flow cytometry) into mitochondria occurred leading to release of cytochrome c (immunofluorescence labeling) into the cytosol and subsequent appearance of apoptotic sub-G1 population (flow cytometry). MEL and its metabolites prominently counteracted these perturbations in both cell lines by 32% ($P < 0.001$, NHEK) and 19% ($P < 0.001$, HDF) decrease of sub-G1. Comparatively, compounds protected mitochondria by maintaining synthesis of ATP by 16% ($P < 0.001$, NHEK) and 14% ($P < 0.001$, HDF). These results suggest and add to our previous investigations with melatonin that also its metabolites AFMK or AMK prominently play a role as strong antioxidative compounds simultaneously preventing serious disturbances within bioenergetics of the cell.

P080

UVR-induced structural and functional alterations are attenuated by the melatonin metabolites AFMK and AMK in human ex vivo full skin

T. W. Fischer, K. Kleszczynski, M. Stegmann, N. Kruse and D. Zillikens *University of Luebeck, Department of Dermatology, Allergology and Venerology, 23538 Luebeck, Germany*

Human skin, the barrier to the environment, plays a crucial role in the regulation of whole-body homeostasis including the mechanical (physical barrier) and functional (immune and antioxidative system, pigmentation) defense against life-long exposure to environmental stressors. One of these stressors is ultraviolet radiation (UVR), and exposure of the skin to UVR leads to short term responses (erythema, sunburn and suntan) as well as long term effects including photoaging and skin cancer. Melatonin (MEL, N-acetyl-5-methoxytryptamine) and its main kynurenic metabolites, i.e. AFMK (N1-acetyl-N2-formyl-5-methoxykynuramine) and AMK (N1-acetyl-5- methoxykynuramine) have recently been shown to significantly enhance epidermal differentiation of human ex vivo skin. One main external stressor that impairs also skin homeostasis and barrier function is ultraviolet radiation (UVR), and melatonin has earlier been shown to be one of the most potent protective agents to counteract UVR-induced oxidative damage by building the melatoninergic antioxidative system of the skin. In this study, we investigated the UV-protective effects of its metabolites, AFMK and AMK, with regard to UVR-induced structural and functional alterations within human epidermis in an ex vivo full skin model. Skin was irradiated with the UV dose of 300 mJ/cm^2 (UVB/A) versus sham-irradiated control (0 mJ/cm^2) in comparison to skin pre-incubated with AFMK and AMK (10^{-3} M) for 1 h prior to UVR exposure. Skin samples were cultured in time-dependent manner after irradiation (0, 24, 48 h post-UVR). Our results showed that UVR significantly induced formation of sunburn cells (SBs) directly (0 h) post-UVR, while presence of AFMK and AMK led to relative 31% (AFMK, $P < 0.05$) and 54% (AMK, $P < 0.05$) decrease of SBs 24 h post-UVR. Subsequent analysis revealed that AFMK and AMK prominently decreased cleavage of Casp-3 by 77% and 49% ($P < 0.01$), respectively, at the same time point. Furthermore, a similar pattern of regulation was observed regarding the induction of heat shock protein 70 (Hsp70). The tested compounds down-regulated its positivity by 77% (AFMK) and 49% (AMK) ($P < 0.01$). Finally, analysis of epidermal differentiation was carried out by using the key markers of non-differentiating (proliferating) basal layer keratinocytes (cytokeratin-14; K14) and keratinocytes of the differentiating spinosum (cytokeratin-10; K10) and granulosum (involucrin; IVL) layer, showing enhancement of all three parameters by AFMK and AMK. In conclusion, it can be claimed that the melatonin metabolites AFMK and AMK may play a crucial role in maintaining structure and integrity of human epidermis under UVR-induced stress conditions.

P081

Inhibition of NADPH oxidase activity or suppression of Nox4 counteracts TGFbeta1- mediated activation of human dermal fibroblasts *in vitro* and attenuates experimentally induced skin fibrosis *in vivo*

H. Dosoki[1,2], A. Stegemann[1], M. Taha[3], H. Schnittler[3], T. Luger[1], K. Schröder[4], H. Distler[5], C. Kerkhoff[6] and M. Boehm[1] *[1]University of Muenster, Department of Dermatology, Muenster, Germany; [2]University of Alexandria, Department of Botany and Microbiology, Alexandria, Egypt; [3]University of Muenster, Department of Anatomy, Muenster, Germany; [4]Institute of Cardiovascular Physiology, Goethe-University, Frankfurt, Germany; [5]University of Erlangen-Nuremberg, Department of Internal Medicine and Institute for Clinical Immunology, Erlangen, Germany; [6]University of Osnabrück, Department of Biomedical Sciences, School of Human Sciences, Osnabrück, Germany*

Systemic sclerosis (SSc) is a complex autoimmune disease with an incompletely understood pathogenesis that involves vascular damage, autoimmune-mediated inflammation and fibrosis. Transforming growth factor-beta1 (TGF-β1), a master regulator of the latter processes as it induces activation of fibroblasts. We hypothesized that (1) distinct NADPH oxidase (Nox) isoforms, especially Nox4, may regulate TGF-β1-mediated activation of human dermal fibroblasts (HDFs), (2) that Nox4 expression is present in SSc fibroblasts, and (3) that suppression of NADPH activity or Nox4 attenuates experimentally induced skin fibrosis. Employing a detailed expression analysis of all Nox isoforms and adaptor proteins at RNA and protein level we found that Nox4 is the major Nox isoform expressed by HDFs. Stimulation of normal HDFs with TGF-β1 resulted in a time- and dose-dependent induction of Nox4 at mRNA and protein levels. This effect was mechanistically dependent on SMAD signaling and was also associated with increased NADPH activity. Immunofluorescence analysis using laser confocal microscopy studies further revealed that Nox4 localizes to the endoplasmic reticulum as demonstrated by double staining with protein disulfide isomerase. Nox4 expression was maintained in SSc fibroblasts. Interestingly, pharmacological inhibition of Nox enzyme activity by diphenyleneiodonium (DPI), a Nox inhibitor, not only suppressed TGF-β1-mediated expression of collagen type I but also induction of both α-smooth muscle actin and fibronectin 1, two established myofibroblast markers. This finding was confirmed in fibroblasts from mice with targeted disruption of the Nox4 as well as in HDFs treated with Nox4 siRNA. Finally, using the bleomycin mouse model of SSc, we found that pharmacological inhibition of NADPH activity by DPI or *in vivo* treatment of mice with Nox4 siRNA lead to significantly reduced collagen content, skin fibrosis and expression of myofibroblast markers. Our findings show that Nox4 is a key intracellular mediator of fibroblast activation. Moreover, targeting Nox4 may be a novel therapeutic strategy for the treatment of fibrotic skin diseases such as SSc.

Dermatopathology

P082

Micro RNAs as a disease modifier in recessive dystrophic epidermolysis bullosa

M. Wimmer[1], R. Zauner[1], T. Lettner[1], N. Niklas[2], J. Reichelt[1] and V. Wally[1] *[1]EB Haus Austria, Research Program for Molecular Therapy of Genodermatoses, Department of Dermatology, University Hospital of the Paracelsus Medical University Salzburg, Austria; [2]Red Cross Transfusion Service for Upper Austria, Linz, Austria*

Recessive dystrophic epidermolysis bullosa (RDEB) is a rare genetic skin disease. RDEB patients suffer from severe blistering after minor trauma, caused by mutations in COL7A1. COL7A1 encodes the extracellular matrix protein type VII collagen which forms anchoring fibrils, connecting the epidermis with the dermis. 87% of RDEB patients develop a particularly aggressive and extremely fast progressing form of squamous cell carcinoma (SCC) before the age of 45, which is the main reason for premature death. Until now, it is not fully understood why these SCCs are much more aggressive than non-EB SCCs.

There is ample experimental data that micro RNAs (miRNAs) act as epigenetic regulators of various oncogenes and tumour suppressors and play an important role in tumour development and progression. Homo sapiens (hsa-)miR711 is encoded within exon 62 of COL7A1. Bioinformatic predictions based on sequence analysis revealed approximately 200 different mRNAs as potential targets of hsamiR711, several of which are associated with tumour development and progression. We performed semi-quantitative RT-PCR on a set of selected disease relevant mRNA targets in combination with TaqMan PCR of hsa-miR711 to assess putative correlation.

In an additional experiment we performed next-generation sequencing of biologically relevant miRNAs co-immunoprecipitated with the RISC component AGO2 to obtain a more comprehensive picture of the regulatory target network of miRNAs in dermal fibroblasts. We sequenced the isolated miRNA of two RDEB patient fibroblast cell lines and a wildtype control and identified 564 and 621 distinct, annotated miRNAs as well as 119 and 229 cell line specific candidates. Of those 181 and 235 were either two fold down- or up-regulated in comparison to wildtype control. Their impact on disease progression will be evaluated in further studies. This approach will allow us to reveal differentially expressed regulatory miRNA – mRNA networks comparing RDEB patients to wildtype control cell lines. The resulting data provides new insights into the molecular mechanisms invoked in RDEB SCC development with future perspectives on new therapy targets.

P083

Loss of inter-alpha-trypsin inhibitor heavy chain 5 (ITIH5) negatively affects skin structure and barrier function of knockout mice

S. Huth[1], R. Heise[1], C. S. Vetter-Kauczok[2], C. Skazik-Voogt[1], Y. Marquardt[1], K. Czaja[1], E. Dahl[3], M. Leverkus[1] and J. M. Baron[1] *[1]University Hospital of the RWTH, Aachen, Germany, Department of Dermatology and Allergology, Aachen; [2]Julius-Maximilians-University, Wuerzburg, Germany, Department of Dermatology, Wuerzburg; [3]University Hospital of the RWTH, Aachen, Germany, Institute of Pathology, Aachen*

We recently characterized ITIH5, a member of the inter-alpha-trypsin inhibitor heavy chains (ITIHs), as the major ITIH family member of the skin. Since the function of ITIH5 in skin is unknown we started to investigate the role of ITIH5 in skin by establishing a new Itih5$^{-/-}$ mouse model. Interestingly, the skin of Itih5$^{-/-}$ mice as well as corresponding *in vitro* 3D skin equivalents exhibited structural abnormalities. In both models a significantly reduced epidermal thickness and absence of a stratified structure as well as a complete lack of the stratum corneum was observed. In consideration of these disturbances we started to assess the consequences of a functional loss of ITIH5 on the skin barrier. Real-Time PCR analyses of Itih5$^{-/-}$ skin equivalents exhibited lower expression levels of the two epidermal barrier molecules filaggrin and involucrin. These findings were strengthened by the outcome of a toluidine blue assay which indicated impairments in the outside-in barrier of Itih5$^{-/-}$ mice. In addition, using a Van-Gieson staining we detected different extracellular matrix (ECM) structures in skin equivalents of Itih5$^{-/-}$ and wild type mice. First results indicate a mechanistic link between the ability of ITIH5 to stabilize the ECMcomponent hyaluronan (HA) and the impaired ECM structure if ITIH5 is lacking.

Taken together, our experiments revealed to our knowledge for the first time a strong involvement of ITIH5 on skin structure and barrier function. In consideration of our observations we assume that ITIH5 could be a novel key player in skin barrier formation. Unrevealing the pathway by which ITIH5 affects skin morphology and barrier function will be the next step and possibly opens new strategies for therapeutic interventions aimed at restoring a dysfunctional skin barrier.

P084

Free Fatty Acid Receptors- possible markers in melanoma?

J. Kleemann, K. Haerle, K. Steinhorst, I. Hrgovic, J. Ter-Nedden, R. Kaufmann, S. Kippenberger and M. Meissner *Klinikum der Goethe-Universität, 60590 Frankfurt am Main, Germany*

The correlation between UV radiation of the skin and melanoma incidence is well established by now. Interestingly, new epidemiologic data suggests also a correlation to an increased body mass index (BMI) pointing to metabolic trigger factors. Interestingly, fatty acids can act directly as signaling molecules in cells via free fatty acid receptors (FFAR), which are members of the G-protein coupled receptor-family. Inspired by studies carried out in colon and prostate carcinoma we hypothesize that FFAR4 (GPR120) and FFAR1 (GPR40) might play a role in human skin melanoma.

Hence, the present study investigates the expression of FFAR1 and FFAR4 in paraffin-embedded tissue section of histologically confirmed nevi, primary melanoma and melanoma metastasis. Normal human skin served as control. The staining was evaluated by three trained investigators and independently scored on a 0–4 point scale.

Preliminary data indicate a distinct higher expression of FFAR1 and FFAR4 in primary melanoma and melanoma metastasis compared to nevi and normal human skin. As secondary finding strong FFAR4 signals were also found in sebaceous and sweat glands. Of note, FFAR1 staining was negative for skin annexes.

Our results point to a functional role of fatty acid receptors in melanoma. Therefore, it could be speculated that targeting these receptors may provide a novel therapeutic option. Further investigations are necessary to evaluate the specific role of lipid signaling in melanoma.

P085

IL-36gamma / IL1-F9 immunohistology identifies psoriasis among erythroderma patients

J. Braegelmann, C. Braegelmann and J. Wenzel *University Hospital Bonn, Dermatology, Bonn*

Erythroderma is a rare but severe dermatological phenomenon caused by different diseases. The aim of this study was to investigate the effectiveness of immunohistological IL-36γ staining in the diagnosis of

psoriasis-based erythroderma among erythrodermic cases. Biopsies of 46 erythroderma-patients were included and scored according to histological criteria for differential diagnoses of erythroderma. Additionally, immunohistochemical staining of IL-36γ was performed and blindly evaluated. The final diagnosis underlying each erythrodermic case was taken retrospectively from the patients files. Psoriasis patients showed a significantly higher expression of IL-36γ compared to every other dermatosis. IL36γ-expression was the most specific and sensitive single marker among other histological criteria for the identification of psoriasis patients. Our results imply that IL-36γ immunohistology is a valuable marker for the identification of a psoriatic pathogenesis among erythroderma patients. This marker could facilitate diagnoses for erythrodermic patients and thus provide them with a specific therapy at an early stage.

P086

The role of Ngfr expression in the progression of malignant melanoma – a histopathological correlation in human primary melanomas and skin metastases

J. Kohlmeyer[1], J. Landsberg[1], E. Ratering[1] and T. Tüting[1,2] [1]*Laboratory for Experimental Dermatology, University of Bonn, 53127 Bonn, Germany;* [2]*Dermatology, University of Magdeburg, 39120 Magdeburg, Germany*

Despite recent groundbreaking progress in the therapy of advanced disease, malignant melanoma remains a clinical challenge due to its rising incidence and tendency to metastasize early in the course of disease. It is still unclear how differentiation and dedifferentiation processes in melanoma cells contribute to disease progression and metastasation. The recapitulation of embryonic pathways and properties may play an important role in early metastasation and dissemination of tumour cells. In our previous work we could demonstrate that the upregulation of the neural crest marker Ngfr (CD271) in melanoma cells is closely associated with a loss of melanocytic differentiation antigens and recognition by melanoma specific Tcells is critically impaired.

In our current work we addressed the question which role the dedifferentiation of melanoma cells may play in the progression of disease. We hypothesized that the proportion of dedifferentiated Ngfr positive melanoma cells increases from human primay melanomas to skin metastases and postulated that dedifferentiation and expression of Ngfr is associated with a worse prognosis.

To this extent we analyzed 178 primary melanomas and 144 skin metastases by histopathological staining for Ngfr expression and expression of gp100 by HMB45 staining.

The cohort of patients and the corresponding histopathological specimens were derived from the dermatohistopathological data base of the Clinic for Dermatology in Bonn which included melanoma patients from the years 2000–2011. Elegibility criteria included primary treatment in-house and availability of sentinel lymph node diagnostics.

We could show in a representative cohort of patients that the expression of the melanocytic marker gp100 is decreased in skin metastastases when compared to their primary skin tumors. This is associated with an increase in Ngfr positive subpopulations in skin metastases whereas Ngfr expression in primary melanomas is significantly lower. We could neither show an association between Ngfr expression in primary melanomas and sentinel lymph node metastases nor an effect on overall survival.

We concluded that dedeifferentation of melanoma cells and the appearance of Ngfr-expressing subsets may be a common event in the course of the disease from primary tumor to skin metastases. More insights on the molecular level are needed to determine how they affect metastasation and disease progression.

P087

Identification of functional microRNA – mRNA regulatory modules in recessive dystrophic epidermolysis bullosa

R. Zauner, M. Wimmer, T. Lettner, J. Reichelt and V. Wally *Laboratory for Molecular Therapy, EB House Austria, EB House Austria, University Hospital of Dermatology, Paracelsus Medical University Salzburg, Austria, 5020 Salzburg*

Recessive dystrophic epidermolysis bullosa (RDEB) represents one of the most devastating subforms of a family of rare genetic diseases that are phenotypically manifesting in blister formation and erosions of the skin and mucous membranes. RDEB is caused by mutations in the COL7A1 gene, encoding type -VII collagen, which forms anchoring fibrils connecting the dermis to the epidermis. Missing or defective type -VII collagen impairs the structural integrity at the dermal-epidermal junction, which leads to blister formation sub lamina densa after mild trauma. RDEB patients are highly prone to develop life threatening squamous cell carcinomas (SCCs) with high incidence and a more aggressive progression compared to non- EB SCCs. The pathomechanisms of EB-related SCC development are currently discussed controversially. Chronic wound healing, inflammation, loss of extracellular matrix components and aberrant signalling are considered to be major factors of carcinogenesis in RDEB patients.

MicroRNAs are small 18–23 nt long non-coding RNAs that promote posttranscriptional regulation of mRNAs via the RNA induced silencing complex (RISC). This results in either transcriptional repression and mRNA decay or up-regulation of gene expression. Increasing evidence demonstrates the many important roles of microRNAs in regulating various biological processes and especially their contribution to cancer development and progression.

In this study we performed Affymetrix GeneChip miRNA 4.0 and Human Gene 2.0 ST microarray analysis in order to determine the microRNA and mRNA expression profiles of a RDEB SCC cell line compared to non-SCC RDEB patient kerationcyte line and wildtype control. We applied a comprehensive and integrative bioinformatics approach on the resulting differential expression datasets by integrating computational microRNA target predictions from miRrecords, miRTarBase and Tarbase, followed by biological functional annotation and enrichment analysis via retrieving the DAVID knowledgebase. Our visualization and comparison using BACA package for R predicts an RDEB-SCC specific microRNA-mRNA interaction network. This interaction network provides us with new insights into the pathomechanisms of RDEB related SCC development and will allow us to identify new therapeutic targets for future treatment options.

P088

The LRIG family – regulators of ERBB signaling in skin during development, homeostasis and tumorigenesis

C. Hoesl, M. R. Schneider, E. Wolf and M. Dahlhoff *LMU München, Institute of Molecular Animal Breeding and Biotechnology, Gene Center, 81377 Munich, Germany*

The leucine-rich repeats and immunoglobulin-like domains (LRIG) family includes transmembrane proteins known to be essential regulators of growth factor receptors like the epidermal growth factor receptor (EGFR/ERBB) family. As ERBBs are involved in cell proliferation, differentiation, death, motility, and adhesion, they are very versatile players during processes as development, tissue homeostasis and tumorigenesis. The LRIG proteins are thought to regulate ERBBs and also other receptor tyrosine kinases. However, the role of LRIG proteins in the different feedback loops are not understood yet and require further studies.

Considering that the LRIG proteins are expressed in the epidermis and that ERBB receptors play an essential role in epidermal physiology and pathology, the skin offers an outstanding model to study the ERBB-LRIG-network.

To study the LRIG proteins in more detail, we generated doxycycline-inducible, skinspecific (keratin 5 promoter-directed) transgenic (tg) mouse lines overexpressing LRIG1 or LRIG2 using the TET-OFF system. As it is known that LRIG1-knockout mice develop a hyperplasia of the epidermis and psoriasis due to a hyperactive EGFR receptor, we anticipated a thinner epidermis in LRIG1 tg mice. Surprisingly, we could not obtain LRIG1 overexpressing mice in the expected rates. Only 9% (6/65) of all born mice were both LRIG1 and tTA positive and 83% (5/6) of these mice died immediately after birth. Only one double transgenic mouse survived for 13 days. The hair coat development of this animal was delayed and it showed reduced hair growth, hyperkeratosis, utricle development and a thicker epidermis compared with control siblings. The latter alteration was accompanied by an increased loricrin positive epidermal layer. Initial investigations in LRIG1 tg mice at day P0 suggest that the loricrin positive epidermal layer was increased in this animals. To study the phenotype of LRIG1 tg also in older animals, we suppressed the LRIG1 overexpression until birth by doxycycline treatment of pregnant females. Induction of the LRIG1 expression after birth by discontinuing doxycycline application enables survival of LRIG1 transgenic mice. These mice showed alopecia from three months of age with a significant thicker epidermis and utricles.

The intracellular domain of LRIG2 differs strongly from that of LRIG1, and it seems that LRIG2 has a major role in cancer biology. Transgenic mice overexpressing LRIG2 show no histological phenotype and the development of these mice seems to be unaffected. Interestingly, LRIG2 KO mice, also generated in our lab, exhibit a significantly thinner epidermis (10.9 ± 0.69 vs. 8.9 ± 0.24 μm; $P = 0.034$).

LRIG2 overexpression in different human cancer types, like glioblastoma or nonsmall cell lung cancer, is often connected with a poor prognosis. Therefore, we anticipated that LRIG2 overexpressing mice would reveal a phenotype under pathological conditions, like chemically-induced skin carcinogenesis or UVB irradiation. For the latter study, LRIG2 transgenic mice and controls were irradiated with 200 mJ/cm² UVB, and their skin was investigated for sunburn cells, increased TRP53 activation and DNA strand brakes. Our new genetically modified mouse models will help to better understand the function of LRIG proteins during skin homeostasis and pathology.

P089

Pathogenicity and biomarkers of the IL-17 pathway in psoriasis

K. Wolk[1,2], E. Witte[1], D. Christou[1], K. Witte[1,2], S. Philipp[1], G. Kokolakis[1], H. Volk[2,3], W. Sterry[4] and R. Sabat[1,5] [1]*University Hospital Charité, Psoriasis Research and Treatment Center, 10117 Berlin, Germany;* [2]*University Hospital Charité, Berlin-Brandenburg Center for Regenerative Therapies, 10117 Berlin, Germany;* [3]*University Hospital Charité, Institute of Medical Immunology, 10117 Berlin, Germany;* [4]*University Hospital Charité, Department of Dermatology and Allergy, 10117 Berlin, Germany;* [5]*University Hospital Charité, Research Center Immunosciences, 10117 Berlin, Germany*

Psoriasis is a chronic inflammatory skin disease affecting about 15 million people in the European Union alone. The cutaneous alterations include a distinct infiltration of immune cells, massively thickened epidermis, and an increased capillary growth. Furthermore, psoriasis is associated with joint and metabolic alterations. Recent clinical trials convincingly demonstrated an essential role of IL-17A in the psoriasis pathogenesis of most affected patients. However, the exact pathogenetic action of IL-17, the elements of the IL-17 pathway, and blood biomarkers indicating a high cutaneous IL-17 activity are currently unknown.

We tried to answer these questions with a translational approach, which included analyses of skin and blood samples as well as experiments with keratinocytes, epidermis models, and isolated immune cells. To search for elements of the IL-17 pathway in psoriasis, we first individually quantified the expression of a broad range of molecules in psoriatic lesions and tested them for statistical correlations with IL-17A. Among others, we found a positive relationships with IL-1beta, IL-17F, IL-21, IL-36, EBI3, and specific chemokines. Accordingly, we show that IL-1beta is an essential mediator for Th17-cell differentiation, which – beside IL-17A – produce IL-17F, IL-22, and IL-21. In epidermis models, IL-17A did not cause morphological psoriatic-like alterations but elevated the expression of IL-36 and EBI3. Following *in vitro* analyses suggested the existence of a novel cytokine containing EBI3, whose biology we are addressing in current experiments. Furthermore, in primary keratinocytes and epidermis models, IL-17A induced a specific pattern of chemokines, which is supposed to mainly attract Th17-cells and neutrophilic granulocytes into the psoriatic skin, therefore creating a positive feedback loop.

A further prominent positive relationship in psoriatic skin was observed between IL-17A and beta-defensin 2 (BD2). Accordingly, IL-17A strongly induced BD2 in primary keratinocytes and epidermis models. Subsequently, we found highly elevated BD2 blood levels in psoriasis patients compared to healthy participants. These levels correlated with disease severity but not with psoriasis duration or age at onset. Interestingly, BD2 blood concentrations did not correlate with metabolic alterations or adipokine blood levels of psoriasis patients suggesting that the IL-17A pathway does not play an important role their endocrine or cardiovascular alterations. In contrast, BD2 correlated with CCL2 blood levels. CCL2, known as a chemokine for attracting several immune cell populations into the skin, also plays a role in osteoclast differentiation. CCL2 might therefore be the link between skin inflammation, IL-17 pathway, and joint alteration in psoriasis. So far, we did not find any hints for a direct involvement of the IL-17 pathway in altered angiogenesis.

In summary, our results suggest that the IL-17 pathway in psoriasis comprises IL-1beta, IL-17, IL-21, IL-36, an EBI3-containing novel member of IL-12 family, BD2, and chemokines, and its activity might be assessed by BD2 blood levels. IL-17A seems to directly support skin infiltration of immune cells, whose mediators cause hyper-proliferation and altered differentiation of keratinocytes.

P090

A crucial role of MMP8 in Acne inversa

A. Tsaousi[1,2], E. Witte[1,2], K. Witte[2,3], H. Röwert-Huber[4], H. Volk[3,5], K. Wolk[2,3], W. Sterry[4], S. Schneider-Burrus[4] and R. Sabat[2,6] [1]*University Hospital Charité, Interdisciplinary Group of Molecular Immunopathology, Dermatology/Medical Immunology, 10117 Berlin, Germany;* [2]*University Hospital Charité, Psoriasis Research and Treatment Center, 10117 Berlin, Germany;* [3]*University Hospital Charité, Berlin-Brandenburg Center for Regenerative Therapies, 10117 Berlin, Germany;* [4]*University Hospital Charité, Department of Dermatology and Allergy, 10117 Berlin, Germany;* [5]*University Hospital Charité, Institute of Medical Immunology, 10117 Berlin, Germany;* [6]*University Hospital Charité, Research Center Immunosciences, 10117 Berlin, Germany*

Acne inversa (AI; also referred to as Hidradenitis suppurativa) is a chronic, recurrent inflammatory skin disease with common onset in the second or third decade of life. It mainly affects the intertriginous skin of perianal, inguinal, and axillary sites of the body and leads to painful and disabling skin lesions including inflamed nodules, abscesses and fistula with foul-smelling secretion. Compared to some other dermatoses, AI shows significantly greater quality-of-life impairment and is frequently associated with metabolic alterations that might increase the risk of cardiovascular disorders and reduce the life expectancy. The AI pathogenesis is still unknown. In order to change this, we individually quantified the expression of a broad range of mediators and other molecules in AI lesions. These analyses revealed an immune dysregulation with simultaneous strong expression of pro-inflammatory cytokines like IL-1β and TNF-α and anti-inflammatory cytokines like IL-10. Moreover, one of the molecules with strongest differential expression between AI lesions and healthy skin was the enzyme matrix metalloproteinase 8 (MMP8). MMP8 is specialized in the degradation of extracellular matrix components, matching very well the destruction of the skin architecture observed in AI lesions. During inflammation, MMP8 was known so far to be secreted by neutrophilic granulocytes, whose presence was observed in AI lesions. Additionally, we demonstrated that also fibroblasts but not keratinocytes expressed MMP8 after stimulation with pro-inflammatory cytokines. The high lesional MMP8 levels were accompanied by elevated blood MMP8 levels. Importantly, these blood levels

positively correlated with disease severity assessed by Sartorius score, especially with the number of regions with inflammatory nodes and fistulas, but not with scars. In contrast to disease severity, there was no correlation between MMP8 and age, AI duration, or age at AI onset. Additionally, MMP8 levels positively correlated with TNF-α levels in blood, supporting the idea that MMP8 indicates the active inflammatory process. Very recently, MMP8 has been shown to also degrade apolipoprotein A-I, the major structural protein component of HDL particles. In our study we found a significant negative correlation between MMP8 and HDLcholesterol levels, suggesting a contributory role of MMP8 in the pathogenesis of cardiovascular disorders in AI patients. In summary, we demonstrate elevated MMP8 levels in AI lesions, suggest their role in skin destruction and metabolic alterations observed in these patients, and recommend the use of MMP8 as a blood biomarker for the objective assessment of AI disease activity.

P091

Enhanced sensitivity of TREX1-deficient cells to cold and UV-irradiation predisposes to autoimmunity

F. Schmidt[1], N. Berndt[1], N. Zimmermann[1], M. Merkel[1], A. Lüth[1], P. Knuschke[1], M. Lee-Kirsch[2] and C. Günther[1] *[1]Technical University of Dresden, University Hospital Carl Gustav Carus, Department of Dermatology, 01307 Dresden, Germany; [2]Technical University of Dresden, University Hospital Carl Gustav Carus, Department of Pediatrics, 01307 Dresden, Germany*

The exonuclease TREX1 safeguards the cell against DNA accumulation in the cytosol and thereby prevents innate immune responses and autoimmunity. Mutations impairing the function of TREX1 lead to type I interferon induction by cell intrinsic innate immune activation. The encephalopathy Aicardi-Goutires syndrome is one of the associated disease phenotypes, characterized by symptoms of autoimmune disease, familial chilblain lupus and systemic lupus erythematosus. Lupus patients with TREX1 mutation were reported to be sensitive to the environmental trigger factors cold and sun light which can induce disease flares. In order to understand how these external triggers lead to disease exacerbation, we analyzed patient fibroblasts for reactive oxygen production, DNA damage response and type I interferon production after exposure to cold or UV-irradiation. We found that TREX1-deficient cells produce enhanced reactive oxygens after cold exposure or UVirradiation. In line with this finding, TREX1-deficient fibroblasts showed enhanced DNA damage and a stronger elevated DNA damage response compared with wildtype cells. This was associated with increased and more sustained upregulation of type I interferon production after challenge with the viral mimic poly(I:C) and solarsimulated UV-irradiation. In conclusion, we showed that TREX1-deficient cells show increased ROS production upon environmental triggers which explains tissue and DNA damage in patients with TREX1-associated lupus erythematosus. Unrestricted DNA damage repair intermediates could trigger type I interferon production and predispose for the increase in type I interferon expression after viral infection and concomitant UV-irradiation. Enhanced type I interferons stimulate the immune response and loss of self-tolerance thereby favoring autoimmunity.

Epidemiology

P092

The National Cancer Aid Monitoring (NCAM) on sunbed use: study design and implications

K. Diehl[1], T. Görig[1], E. W. Breitbart[2], R. Greinert[2,3] and S. Schneider[1] *[1]Heidelberg University, Medical Faculty Mannheim, Mannheim Institute of Public Health, Social and Preventive Medicine, 68167 Mannheim, Germany; [2]Arbeitsgemeinschaft Dermatologische Prävention, 20457 Hamburg, Germany; [3]Elbe Kliniken Buxtehude, Centre of Dermatology, 21614 Buxtehude, Germany*

Introduction: There is an increase in the incidence of malignant melanoma, basal cell carcinoma and squamous cell carcinoma in Germany and other developed countries. One reason for this development is a completely avoidable risk factor: the use of sunbeds. In 2009, the International Agency for Research on Cancer (IARC) classified ultraviolet rays of the sun and explicitly those of sunbeds as carcinogenic to humans. In previous studies, i.e., the regional SUN-Study 2008 (Sunbed Use: Needs for Action-Study 2008) and a nationwide representative study (SUN-Study 2012), we revealed the prevalence of sunbed use in Germany and shed light into the motivation for sunbed use and the risk perception among sunbed users and non-users. Based on this, we currently conduct the National Cancer Aid Monitoring (NCAM) on sunbed use.
Methods: NCAM is a longitudinal monitoring instrument focusing on sunbed use, its determinants, and other related topics. Within four waves (2015–2018), an annual telephone survey will be realized. The research design is divided into two parts: (1) 3000 individuals aged 14–45 years will be interviewed each year in a crosssectional representative survey. (2) A cohort of 450 sunbed users will be formed in the first year and will be followed in the subsequent 3 years. The NCAM is funded by the "Deutsche Krebshilfe e.V." from 07/2015 to 06/2019.
Results: The first of the four surveys starts in October 2015. Preliminary results including the current prevalence of sunbed use and its determinants will be presented at the conference.
Discussion : The concept of the NCAM is unique. The outcomes of the representative survey will enable us to investigate the long-term trends in sunbed use. The cohort consisting of current sunbed users makes it possible to investigate sunbed use for an extended period. The results will help to develop targeted campaigns for health promotion and prevention.

P093

Epidemiologic and genetic association between atopic dermatitis, rheumatoid arthritis, inflammatory bowel disease, and type-1 diabetes

H. Baurecht[1], J. Schmitt[2], E. Rodrguez[1], W. Lieb[3], C. Gieger[4], A. Irvine[5], N. Novak[6] and S. Weidinger[1] *[1]University Hospital Schleswig-Holstein, Campus Luebeck, Department of Dermatology, Venereology and Allergy, Kiel; [2]Medizinische Fakultät Carl Gustav Carus, TU Dresden, Center for Evidence-based Healthcare, Dresden; [3]Institute of Epidemiology and PopGen Biobank, University Hospital Schleswig-Holstein, Kiel; [4]Helmholtz Zentrum München, German Research Center for Environmental Health, Research Unit Molecular Epidemiology, Institute of Epidemiology II, Neuherberg; [5]Our Lady's Children's Hospital, Crumlin, Department of Paediatric Dermatology, Dublin; [6]University of Bonn, Department of Dermatology and Allergy, Bonn*

Atopic dermatitis (AD) is characterized by epidermal barrier failure and cutaneous inflammation. Molecular studies suggested shared genetic factors and immunological pathways with other inflammatory diseases as rheumatoid arthritis (RA) and inflammatory bowel disease (IBD), but epidemiological evidence is scarce.
We test the hypothesis that prevalent AD is a risk factor for incident RA and IBD and inversely related to type-1 diabetes (T1D) and investigate RA, IBD, and T1D susceptibility loci in AD.
This cohort study utilized data from German National Health Insurance beneficiaries age 40 or younger (n = 655 815) from 2005 through 2011. Prevalent AD in 2005/2006 was defined as primary exposure, and incident RA, IBD, and T1D in 2007–2011 as primary outcomes. Risk ratios were calculated and established RA, IBD and T1D loci were explored in high density genotyping data.
Patients with prevalent AD were at increased risk for incident RA (risk ratio (RR) 1.72, 95% CI = 1.25–2.37), CD (RR 1.34, 95% CI = 1.11–1.61) and UC (RR 1.25, 95%CI = 1.03–1.53). There was no disproportionate occurrence of known RA, CD, UC or T1D risk alleles in AD.
AD is a risk factor for the development of RA and IBD. The excess comorbidity cannot be attributed to major known IBD and RA genetic risk factors.

P094

Effectiveness of a PCR based general screening at admission to prevent MRSA transmission and to reduce the prevalence rate

S. von Podewils[1], G. Daeschlein[1], T. Bloom[1], H. Haase[1] and M. Jünger[1] *[1]University Medicine Greifswald, Departement of Dermatology, 17475 Greifswald, Germany*

Objective : As a consequence of an outbreak in our dermatology we implemented a PCR based general screening at admission together with strict protective isolation until PCR results were available and investigated the effectiveness of this intervention to prevent MRSA transmission and the impact on prevalence rate.
Methods: After a MRSA outbreak we started a PCR based general admission screening in the dermatologic ward of our University clinic. In short, outpatient patients are screened 14 day before admission by conventional swab microbiology and on admission day by PCR. When positive patients were detected, ambulatory sanitation was started. Swabs were taken of the anterior nares, wounds and any other skin area involved in skin disease. The PCR results were obtained within 2 h and the patients released from protective isolation. Positively tested patients were further isolated and topical decolonization for 7 days was started. Preventive isolation measures were withdrawn after 3 consecutive negative screening samples of all former positive areas. End-points were the number of MRSA patients, the MRSA-rate, the diagnosis-specific burden, the MRSA-prevalence and the incidence density.
Results: During the intervention of 4 years, no further MRSA transmission occurred. 60 patients (1.6%) from overall 3788 patients were found MRSA positive, 56 patients with colonization (93.3%) and 4 with infection (6.7%) – 3 wound infections by HAMRSA and one furunculosis caused by CA-MRSA. After intervention the prevalence decreased from 14.7% to 1.6% and the nosocomial resp. overall incidence density from 12.1 to 0 resp. 19.4 to 1.8. After intervention the MRSA rate (proportion of MRSA per all samples of Staphylococcus aureus)1 decreased from 48% to 16.7%.
Stabilization of MRSA prevalence rate and incidence density occurred not before the 12th month of intervention. From July 2007 no significant ($P > 0.05$) decreasing or increasing appeared.
The prevalence data within the intervention phase did not show a significant increase. The prevalence decreased steadily within one year with some slight variations and follows the prediction model. The prevalence has its highest value in the first month of the outbreak (28%) and decreased to 2% after 56 month of intervention.
The incidence density (nosocomial and non-nosocomial) was maximal during the outbreak reaching 47 in June and decreased significantly after starting the intervention to a mean value of 1.9. We also estimated the upper 95% confidence level to identify significant exceptions which did not appear. The level of MRSA incidence which can be predicted at least without intervention would increase significantly without intervention.
Conclusion: We could demonstrate high prevalence rates in dermatology with a high proportion of acute and chronic wounds. This has led to a severe outbreak touching nearly 50% of the inpatients. Before implementing interventions, every dermatologic unit should collect their own epidemiologic data for individual risk assessment. Together with the associated infection control measures we conclude our PCR-based general admission screening as effective to prevent further nosocomial transmission and to reduce the prevalence of MRSA as risk for transmission and infection.

P095

The impact of gliding on the prevalence of non-melanoma skin cancer and its precursors – a cross-sectional study among male pilots of glider aircrafts in Bavaria

I. Hänsel[1], M. Rotter[2], F. Lauffer[1], T. Biedermann[1] and A. Zink[1] *[1]Department of Dermatology and Allergy, Technische Universität München, Munich, Germany; [2]Institute of Epidemiology II, Helmholtz Zentrum München, Neuherberg, Germany*

Background: One out of three diagnosed cases of cancer is skin cancer with a continuously increasing incidence in the last decades worldwide. The main role in the development of non-melanoma skin cancer (NMSC) and its precursors has solar UV radiation. Pilots of gliding aircrafts are heavily exposed because they are several hundred to thousands meters above the ground during a flight, which can last up to a whole day. At these altitudes they are less protected from UV radiation, which assumedly increases the risk of photo damage of the skin compared to the general population.
Objectives: The aim of this study was to estimate the prevalence of NMSC in male glider pilots and to investigate whether NMSC was associated with gliding.
Methods: The data were collected between May and July 2015 with a cross-sectional study using a self-administered questionnaire and a clinical skin examination of NMSC by a dermatologist among male pilots of gliders in Bavaria, Germany. A random sample of 82 pilots aged 18–83 years old of four large gliding clubs participated. Data were analysed with logistic regression analyses and the associations were expressed as odds ratios (OR) with 95% confidence intervals (CI).
Results: The overall prevalence of NMSC was 49% (40 of 82). For the exposure the ratios were for '11–20 years' gliding experience OR = 1.14 (CI = 0.10–12.71), for '21–30 years' OR = 1.68 (CI = 0.24–11.88), for '31–40 years' OR = 1.40 (CI = 0.22–9.13) higher and for '41 and more years' OR = 0.72 (CI = 0.066–7.71) lower compared with '0–10 years' gliding experience.
Conclusion: The study suggests an association between NMSC and gliding in terms of a higher prevalence. Further studies are needed to strengthen this hypothesis and escpecially to evaluate UV-radiation compared to cosmic radiation as potental risk factors for NMSC in gliding pilots.

P096

Individualized extemporaneous formulations- a frequent choice in dermatologists practice

P. Staubach, S. Salzmann, B. Lang, A. Peveling-Oberhag and S. Zimmer *University Medical Center, Dermatology, 55131 Mainz, Germany*

Background : Due to the wide range of medicinal products in the dermatological field, the need of individualized extemporaneous formulations is often discussed. The use of this individualized medicine leads to an optimized supply in treating patients with active ingredients and galenics. However, there is no existing information about the need, importance and relevance of individualized formulations compared to finished medicinal products in everydays practice. We evaluate the prescription behaviors of physicians in the topic 'topical formulations' since 2011- to investigate the need for individualized prescriptions.
Methods: The analyzed data was compiled by the German Institute for Drug Use Evaluation (GIDE/DAPI). It contains information on the number of individualized prescriptions and the finished medicinal product concerning the ambulant treatment and the compulsory health insurance. Different categories were evaluated: regions (Germany and in detail 2 individual districts Rhineland-Palatinate and Saarland), quarters (2011–2014), specialists (dermatologists, pediatricians, general practitioners) and prescription profiles. The analyses were carried out with the SPSS-Software of IBM.
Results: Data includes information from 4th quarter of 2011 through the 3rd quarter of 2014. The research included 1 912 964 745 prescriptions, dealing with a value of 92 634 644 947 EUR. 1.3% (25 619 489) prescriptions were individualized formulations with a value of 509 529 621 EUR. Concerning the 3th quarter of 2012, more than 1.9 million individual formulations were prescribed in Germany. 53.62% (1 019 456) of those individual formulations were prescribed by dermatologists, 19.04% by general practitioners, 9.23% by pediatricians leading to an average of 245 individual formulations prescribed by one dermatologist, 26 by one pediatician, 9.7 by one general practitioner

during the 3rd quarter of 2012. 3 of 10 prescriptions done by a dermatologist are individual formulations.
Conclusions: Considering our findings, there is a high need for individualized formulations not only in dermatology. More studies have to be done to investigate the individualized formulations by ingredients and amounts at least in dermatology.

P097
Epidemiology and characterization of pediatric psoriasis population

G. Kokolakis[1,2], F. Bachmann[2], K. Stieler[3], R. Sabat[1,2] and S. Philipp[1,2] *[1]Charité – Universitätsmedizin Berlin, Interdisciplinary Group of Molecular Immunopathology, Dermatology/Medical Immunology, 10117 Berlin, Germany; [2]Department of Dermatology, Venerology, and Allergology, Charité – Universitätsmedizin Berlin, Psoriasis Research and Treatment Center, 10117 Berlin, Germany; [3]Department of Dermatology, Venerology, and Allergology, Charité – Universitätsmedizin Berlin, Clinical Research Center for Hair and Skin Science, 10117 Berlin, Germany*

Characterizing and identifying the special traits of the pediatric psoriasis population has attracted the interest of treating physicians. The early recognition of high need patients and the timely initiation of the proper treatment should be of benefit for the patients. It has been supposed HLA-cw6 is associated with psoriasis. However, we lack data in pediatric psoriasis. Moreover, metabolic syndrome has strongly been proven to correlate with psoriasis.
A prospective register has been established in the interdisciplinary consultation hour for pediatric psoriasis in the Clinic of Dermatology, Venerology and Allergology, Charité Universitätsmedizin Berlin. Epidemiological data, metabolic syndrome parameters, gene mutation of HLA-cw6 as well as disease activity and applied therapies over two years have been captured.
Mean age of the population is 11.5 ± 4.6 years at the date of inclusion. BMI of the included patients was at the average 19.9 ± 4.6. Patients with plaque-type, nail, pustular psoriasis as well as psoriasis inversa are included. Among them approximately 26% also suffer from psoriatic arthritis. More than two thirds of the examined population received at the inclusion visit systemic treatment. Up to now analyzed data showed a positivity of HLA-cw6 of approximately 66% of the patients. Interestingly, HLA-cw6 positivity does not seem to correlate with the psoriasis phenotype.
Emerging from the current results we can conclude that a subgroup of children with psoriasis might be a demanding population for systemic treatment. Obesity may not be the main metabolic disease of the pediatric population but further parameters of the metabolic syndrome have to be analyzed. HLA-cw6 might be a promising genetic marker for children predisposed to develop severe psoriasis. Further collection of data will elucidate the unique characteristics of pediatric psoriasis.

P098
Characterisation of 242 patients with bullous pemphigoid with and without neurological disorder

M. Kolesnik[1], A. Ambach[1], A. Lux[2], D. Reinhold[3], B. Bonnekoh[1] and H. Gollnick[1] *[1]Otto-von-Guericke-University Magdeburg, Clinic for Dermatology and Venereology, 39120 Magdeburg, Germany; [2]Otto-von-Guericke University Magdeburg, Department for Biometrics and Medical Informatics, 39120 Magdeburg, Germany; [3]Otto-von-Guericke-University Magdeburg, Institute for Molecular and Clinical Immunology, 39120 Magdeburg, Germany*

Patients with bullous pemphigoid (BP) suffering from a neurological diseases (ND) are a well known clinical phenomenon. However, if this is more than a coincidence or related to the higher age of BP patients, if comorbidities are involved and/or comedication or if expression of BP autoantigens in the brain are of pathogenetical relevance is completely unclear.
To shed light on these questions, 144 BP-patients with ND (BP + ND) and 98 BPpatients without ND (BP-ND) as well as 100 age- and sex-matched subjects suffering from herpes zoster (HZ) as a control group were included in a retrospective monocentric study.
Comparing cases to controls, dementia and stroke were significantly associated with BP (38.4% and 28.1% vs 11%; $P \leq 0.001$). No significant correlation, however, was found with M. Parkinson, epilepsy, schizophrenia and/or multiple sclerosis. In contrast, BP correlated significantly with diabetes mellitus (DM). DM prevalence was twice as high in BP as compared to HZ-controls (40.1% vs 20%; $P = 0.01$).
BP + ND patients were older than BP patients without ND (82.5 vs 76.6 years old, $P < 0.001$), with female preponderance in both groups. Peripheral eosinophilia was significantly more often observed in BP + ND as compared to BP-ND (63.6% vs 48.9%, $P = 0.03$). Autoantibodies (BP180, BP230) and serum levels of total IgE showed no significant difference between both groups. Analysis of comedication revealed a significant increased intake of antipsychotics, anti-Parkinson drugs and anticonvulsant in BP + ND as compared to BP-ND and a significant reduced consumption of angiotensin receptor blockers and oral antidiabetics. Furthermore, chronic intake of loop diuretics was significantly associated with BP as compared to HZ-controls.
These findings confirm an increased prevalence of dementia and stroke in BP patients. However, in our cohort, BP with CNS involvement did not differ clinically or with regard to autoantibodies levels from BP without CNS involvement. There were significant differences between both groups in eosinophilia, age of onset, comorbidities and comedication. Although these differences do not explain sufficiently the association of ND and BP, increased DM-prevalence and reduced consumption of antihypertonics and antidiabetics in the BP + ND group might contribute to the observed increased incidence of stroke and dementia.

Genetics

P099
TALEN-mediated elimination of mutant keratin 14 as a gene therapy for epidermolysis bullosa simplex

M. Aushev[2], C. Mussolino[3], T. Cathomen[3] and J. Reichelt[1,2] *[1]Salzburger Landeskliniken, EB House, Dermatologie, 5020 Salzburg, Austria; [2]Newcastle University, Institute of Cellular Medicine, NE2 4HH Newcastle, UK; [3]University Medical Centre Freiburg, Institute for Cell and Gene Therapy, 79108 Freiburg, Germany*

Epidermolysis bullosa simplex (EBS) is an inherited bullous skin disorder characterised by blistering of the skin after minor trauma. It is caused by heterozygous dominant-negative mutations in the keratin 5 (KRT5) or keratin 14 (KRT14) genes. Mutant keratins integrate into the intermediate filament cytoskeleton impairing filament stability resulting in skin fragility. Currently there is no cure for EBS.
We have previously shown that zinc finger nucleases (ZFNs) can be used to efficiently inactivate a transgene in murine keratinocyte stem cells without impairing the stem cell properties. We aim to develop an ex vivo gene therapy for EBS which uses transcription activator-like effector nucleases (TALENs) to inactivate the mutant KRT14 allele in patient keratinocyte stem cells (KSCs). Correctly modified KSCs will then be grafted back onto the patient's skin.
Our gene therapy approach is being validated using KRT14-specific TALENs and immortalised patient-derived EBS keratinocyte lines carrying distinct KRT14 mutations causing a severe and moderate EBS phenotype respectively. KRT14-specific TALENs were engineered using the Golden Gate assembly method. A T7E1 assay confirmed the ability of KRT14 TALENs to modify their target site. Transfected keratinocytes were clonally expanded and correctly modified clones identified. These clones are currently being analysed biochemically and functionally.

P100
Gene editing of keratinocyte stem cells for a novel ex vivo epidermolytic ichthyosis therapy

O. March[1,2], M. Aushev[2], U. Koller[1] and J. Reichelt[1,2] *[1]Salzburger Landeskliniken, EB House, Dermatology, 5020 Salzburg, Austria; [2]Newcastle University, Institute of Cellular Medicine, NE2 4HH Newcastle, UK*

Epidermolytic ichthyosis (EI) is an inherited skin fragility disorder caused by dominant-negative mutations in either the keratin 1 (KRT1) or keratin 10 (KRT10) genes. As EI is difficult to treat and currently lacks a cure, there is an acute need for novel therapies.
Keratins are expressed in pairs, specifically polymerising to build the intermediate filament cytoskeleton of epithelial cells. Dominant-negative mutant keratins integrate into the cytoskeleton, resulting in fragility and collapse upon mild stress. As is the case in EI, this leads to cytolysis and blistering of the skin. Elimination of these mutant keratins is essential for curation of the disease.
Heterozygous parents of patients with recessive EI, express only one KRT10 allele. Their normal phenotype demonstrates that this is sufficient to support normal skin function.
We are developing an ex vivo gene therapy for EI using transcription activatorlike effector nuclease (TALEN) technology to knockout mutant KRT10 alleles in keratinocyte stem cells (KSCs). TALENs are sequence specific nucleases which can be transfected transiently, so as not to persist in treated cells. The specific genome modifications persist and are passed on to the KSC progeny.
Once proof-of-principle is demonstrated, TALEN technology can be applied to a range of genetic skin diseases.

P101
Photosensitive form of trichothiodystrophy associated with a novel mutation in the XPD gene

B. Brauns[1,5], S. Schubert[1], J. Lehmann[1,5], P. Laspe[1], A. Körner[2], K. Brockmann[3], M. P. Schön[1,4] and S. Emmert[1,5] *[1]University Medical Center Goettingen, Department of Dermatology, Venereology and Allergology, 37075 Goettingen, Germany; [2]DWI, Leibniz Institute for Interactive Materials, 52074 Aachen, Germany; [3]University Medical Center Goettingen, Interdisciplinary Pediatric Center for Children with Developmental Disabilities and Severe Chronic Disorders, 37075 Goettingen, Germany; [4]University Medical Center Goettingen and University of Osnabrueck, Lower Saxony Institute of Occupational Dermatology, 49074 Osnabrueck, Germany; [5]University Medical Center Rostock, Clinic for Dermatology and Venereology, 18057 Rostock, Germany*

An eight weeks-old boy was referred to our clinic with dry, scaly skin on the trunk and dorsal extremities sparing the flexures. In the second year of his life, however, the patient additionally exhibited short, brittle hair and nails, nail dystrophy, photosensitivity, mild delay of motor and speech development, and marked ataxia. He also suffered from frequent febrile infections of the upper respiratory system.
Analysis of the patient's hair by light microscopy revealed transverse fractures (trichoschisis). Alternating dark and light bands of his hair shafts ('tiger-tail' banding) were discovered using polarizing microscopy. Subsequently, amino acid analysis of hydrolyzed hairs showed a markedly reduced cystine content leading to the diagnosis of trichothiodystrophy (TTD) a rare autosomal recessive disorder characterized by sulfur-deficient brittle hair and other neuroectodermal symptoms. Magnetic resonance imaging of the brain revealed mild diffuse T2-hyperintensity of supratentorial white matter consistent with dysmyelination, as described previously in TTD.
We established a fibroblast cell line (TTD5GO) from a skin punch biopsy. After irradiation with 30 J/m^2 UVC, the cells showed a reduced relative post-UV cell survival rate of only 61.4% compared to >75% of normal fibroblasts. As most photosensitive forms of TTD are predominantly caused by defects in the xeroderma pigmentosum group D gene (XPD/ERCC2) rather than by mutations in XPB/ ERCC3 or TTDA, we performed XPD gene sequencing. Indeed, the patient was compound heterozygous for 2 mutations in XPD: c.2164C>T (p.R722W; from father); described at least in 7 patients and known TTD-causing and a novel TTD-associated c.2174C>T mutation (p.A725V; from mother). Interestingly, another amino acid exchange at the same position (p.A725P) has been previously identified as TTDcausing in another patient. Re-introducing wild type XPD cDNA into TTD5GO cells increased their repair capacity three-fold as assessed by host cell reactivation indicating complementation by XPD.
The xeroderma pigmentosum (XP) group D gene is a subunit of the DNA repair/ transcription factor TFIIH. XPD mutations in TTD patients seem to predominantly affect transcription whereas XPD mutations in patients with XP primarily interfere with DNA repair. Therefore, unlike XP patients, patients with TTD are not skin cancer prone and the clinical course is determined by the involvement of other organs and mortality is mainly due to severe systemic infections.

P102
Copy number variants of β-defensin gene towards genetic predisposition for Hidradenitis Suppurativa/Acne Inversa

I. Karagiannidis *Departments of Dermatology, Venereology and Allergology, Dessau Medical Center, 06847 Dessau, Germany*

Hidradenitis suppurativa / acne inversa (HS) is a chronic, inflammatory, recurrent, debilitating skin disease, that usually presents after puberty with painful, inflamed lesions in the apocrine gland-bearing areas of the body, most commonly the axillary, inguinal, and anogenital regions. The pathogenesis of HS remains unknown. Family predisposition is part of the multifactorial process associated with the development of HS generating questions for a possible genetic disease fingerprint. Antimicrobial peptides that are expressed by keratinocytes are part of the innate immune response to skin commensals. One of them, human β-defensin-2 (hBD-2) is encoded from DEFB4, which exists as clusters of copy number variations (CNVs). A prospective case-control, cooperation study of Athens, Greece and Saxony Anhalt, Germany examined CNVs of DEFB4 by the paralog ratio test in the genomic DNA from greek and german patients with HS and controls in two independent cohorts. The CNVs were greater in patients than controls in both studied cohorts. Furthermore, it has been shown that presence of more than 6 CNVs of DEFB4 was linked with susceptibility for HS, milder disease, later onset, lower rate of permanent purulent skin lesions and involvement of a lower number of characteristic skin areas. A genetic trait for susceptibility to HS is provided for the first time and this is confirmed in two independent cohorts.

P103
Increased prevalence of filaggrin deficiency in recessive X-linked ichthyosis

K. Süßmuth[1], E. Rodrguez[2], F. Valentin[1], T. Tarinski[1], H. Traupe[1], I. Hausser-Siller[3], S. Weidinger[2] and V. Oji[1] *[1]University of Muenster, Department of Dermatology, Muenster, Germany; [2]University of Kiel, Department of Dermatology, Kiel, Germany; [3]University of Heidelberg, Department of Dermatology, Heidelberg, Germany*

Recessive X-linked ichthyosis (RXLI) is a keratinization disorder caused by steroidsulfatase deficiency; ichthyosis vulgaris (IV) is due to semidominant mutations of the filaggrin gene (FLG). Clinically it may be difficult to distinguish between the two ichthyoses. Coincidence of RXLI and IV was noted in singular case reports.
We performed a prospective study and analyzed the prevalence of FLG mutations in RXLI.

Diagnosis of RXLI was confirmed in 36 patients. The five most common FLG mutations (R501X, 2282del4, R2447X, S3247X, 3702delG) were analyzed by restriction enzyme and TaqMan allelic discrimination assay.
In the overall group ($n = 36$) we identified 16 patients, who showed clinical or morphological signs of concomitant filaggrin deficiency. FLG mutations were confirmed in eight patients. As such the prevalence of FLG mutation carriers in the RXLI cohort was significantly higher ($P = 0.004$) than in a control cohort of 1377 healthy patients from northern Germany (FLG mutation carriers 22.22% vs 8.42%, $P = 0.004$). One patient with RXLI was compound heterozygous for the mutations R501X and 2282del4 and clinically showed a severe phenotype. Within the RXLI group palmoplantar hyperlinearity was significantly associated with the FLG mutation status ($P = 0.012$). Atopy was highly prevalent in both groups (42.9% vs 50%). The severity of ichthyosis seems to be increased in RXLI with associated FLG deficiency (average ichthyosis score of 1.85 vs 2.25).
In this collection of RXLI patients the FLG mutation frequency was surprisingly high. This may be explained by the higher ichthyosis severity score of patients with RXLI and filaggrin deficiency. Clinically, palmoplantar hyperlinearity appeared as the diagnostic clue for RXLI and IV copresentation. As such our study may lead to a better understanding of the common types of ichthyosis and their differential diagnosis.

P104

Genome-wide association study identifies new susceptibility loci for cutaneous lupus erythematosus

M. Kunz[1], I. R. König[2], A. Ziegler[2], A. Ranki[3], S. Koskenmies[3], A. Rönn[4], J. C. Simon[1], J. Wenzel[5], J. Landsberg[5], T. Zeller[6], R. Gläser[7], A. Kuhn[8] and S. Ibrahim[9] *[1]University of Leipzig, Department of Dermatology, Venereology and Allergology, 04103 Leipzig, Germany; [2]Universitätsklinikum Schleswig-Holstein, Campus Luebeck, 23562 Luebeck, Germany; [3]Helsinki University Central Hospital, Department of Dermatology and Allergology, 00029 Helsinki, Finland; [4]Karolinska University Hospital, Clinical Research Center, 14157 Huddinge, Sweden; [5]University of Bonn, Department of Dermatology and Allergy, 51105 Bonn, Germany; [6]University Heart Center Hamburg, Clinic for General and Interventional Cardiology, 20246 Hamburg, Germany; [7]University of Schleswig-Holstein, Campus Kiel, Department of Dermatology and Allergology, 24105 Kiel, Germany; [8]University Medical Center Mainz, Interdisciplinary Center for Clinical Studies, 55131 Mainz, Germany; [9]University of Schleswig-Holstein, Campus Luebeck, Department of Dermatology, Allergology and Venereology, 23562 Luebeck, Germany*

Cutaneous lupus erythematosus (CLE) is a chronic autoimmune disease of the skin with typical clinical manifestations. While the genetic basis for systemic lupus erythematosus (SLE) has been investigated in more detail in the past, little is known about the genetics of CLE. Here, we genotyped 906 600 single nucleotide polymorphisms (SNPs) in 183 CLE cases and 1288 controls of Central European ancestry. Replication was performed for 13 SNPs in 219 case subjects and 262 controls from Finland. Association was particularly pronounced at 4 loci, all with genome-wide significance (P Ü 5×10^{-8}): rs2187668 (PGWAS = 1.4×10^{-12}); rs9267531 (PGWAS = 4.7×10^{-10}); rs4410767 (PGWAS = 1.0×10^{-9}); rs3094084 (PGWAS = 1.1×10^{-9}). All mentioned SNPs are located within the major histocompatibility complex (MHC) region of chromosome 6 and near genes of known immune functions or associations with other autoimmune diseases such as HLA-DQ alpha chain 1 (HLADQA1), MICA, MICB, MSH5, TRIM39, and RPP21. E.g., TRIM39-RPP21 read through transcript is known mediator of the interferon response, a central pathway involved in the pathogenesis of CLE and systemic lupus erythematosus (SLE). Conditional analyses indicated a dependence of the signals, so that it is, at this stage, difficult to decide whether there are one or several underlying signals. Further studies with larger samples are required to clarify this. Taken together, this genome-wide analysis of disease-association of CLE identified candidate genes and genomic regions that may contribute to pathogenic mechanisms in CLE via dysregulated antigen presentation (HLADQA1), apoptosis regulation, RNA processing and interferon response (MICA, MICB, MSH5, TRIM39, RPP21). These analyses may help in future functional studies on this up to now poorly understood disease.

P105

Single amino acid deletion in kindlin-1 results in partial protein degradation which can be rescued by chaperone treatment

K. Maier[1], Y. He[1], P. R. Eßer[1], K. Thriene[1,2], D. Sarca[1], J. Kohlhase[3], J. Dengjel[1,3], L. Martin[4] and C. Has[1] *[1]Medical Center – University of Freiburg, Department of Dermatology and Venereology, 79104 Freiburg, Germany; [2]University of Freiburg, Freiburg Institute for Advanced Studies, 79104 Freiburg, Germany; [3]Private Practice of Human Genetics, 79100 Freiburg, Germany; [4]Angers University Hospital, Department of Dermatology, 49100 Angers, France*

Kindler syndrome (KS), a distinct type of epidermolysis bullosa, is a rare disorder caused by mutations in FERMT1, encoding kindlin-1. Most FERMT1 mutations lead to premature termination codons and absence of kindlin-1. Here we investigated the molecular and cellular consequences of a naturally occurring FERMT1 mutation, c.299_301del resulting in a single amino acid deletion, p.R100del. The mutation led to a 50% reduction of FERMT1 mRNA and 90% reduction of kindlin-1 protein in the keratinocytes derived from the patient, as compared to the control cells. Low levels of wild type or p.R100del mutant kindlin-1 were sufficient to improve the cellular phenotype in respect of spreading and proliferation as compared to kindlin-1 negative keratinocytes. The misfolded p.R100del mutant was lysosomally degraded and launched a homeostatic unfolded protein response. Sodium-phenylbutyrate significantly increased kindlin-1 mRNA and protein levels and the area of mutant cells, acting as a chemical chaperone and probably also as a histone deacetylase inhibitor. The study of this hypomorphic mutation has therapeutic relevance. It provides the first evidence that low amounts of kindlin-1 improve the epidermal architecture and KS cellular phenotype and proposes a personalized chaperone therapy for the patient.

P106

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

S. Giovannini[1], Y. Kamenisch[2], L. Weibel[3], T. Sinnberg[1], N. Kobert[3], B. Schittek[1], L. E. French[3], M. Röcken[1] and M. Berneburg[2] *[1]Eberhard Karls University, Department of Dermatology, 72076 Tuebingen, Germany; [2]Universitätsklinikum Regensburg, Department of Dermatology, 93053 Regensburg, Germany; [3]University Childrens Clinic, Childrens Hospital Zurich, 8032 Zurich*

Prophylactic protection of patients with severe immunosuppression is important to shield the patient from opportunistic fungal infections.
Patients treated with a broad spectrum antimycotic drug can develop adverse effects such as phototoxicity followed by pigmentary changes and the development of ultraviolet radiation (UV) associated non melanoma skin tumors. Thus, patients closely resemble the phenotype of the progeroid disorder xeroderma pigmentosum (XP), known to be caused by a defect in the DNA repair mechanism nucleotide excision repair (NER). So far the underlying molecular mechanisms by which this drug leads to the XP-like clinical phenotype have not been clarified. Therefore, we investigated if the antimycotic drug leads to a reduction of DNA repair and increases DNA damage. We found that long term treatment lead to suppression of unscheduled DNA synthesis as well as increased comet formation while double strand breaks were not significantly induced. Importantly repair suppressive effects were transient since removal lead to normalization of all repair associated parameters. Furthermore, compound treatment did not cause significant transcriptional regulation of mRNA levels of NER proteins such as XPA – G, ERCC1, BRCA1, BRCA2 and RAD23 A/B and of DNA damage signaling factors (ATM and ATR). Furthermore, we found a higher level of Mdm2, XPB and XPD proteins in complex with p53 upon AD treatment and it is known that p53 is involved in chromatin remodeling during damage processing. Interestingly electronmicroscopy showed antimycotic drug induced changes in Chromatin density and further analysis revealed presence of the antimycotic drug in chromatin. When exposed to the compound cells also did not show cell cycle arrest even in the presence of DNA damage but proliferated similar to untreated controls.
Taken together these results indicate that the broad spectrum antimycotic could suppress NER, increase DNA damage and thus, within months lead to photosensitivity, pigmentary changes and ultimately skin tumors.

P107

Systematic identification and characterization of novel human adiposeassociated genes encoding membrane and secreted proteins

B. A. Buhren[1], H. Schrumpf[1], B. Homey[1], A. Zlotnik[2], P. Hevezi[1] and P. A. Gerber[1] *[1]Medical Faculty, University of Duesseldorf, Department of Dermatology, 40225 Duesseldorf, Germany; [2]University of California, Irvine, Physiology & Biophysics, 92697-4560 Irvine, CA, USA*

Recently, we used the BIGE database to identify genes which are highly expressed in human skin and we have used a similar approach to find adipose-associated genes (AAGs). Through bioinformatics analyses of a human gene expression database representing 105 different tissues and cell types, we identified 10 genes that are expressed exclusively at high levels in AAGs. Given its increasing significance in human disease, characterization of adipose tissue specifically by identifying genes which are highly and predominantly expressed by the tissue is of primary importance. The high levels of adipose-associated expression for 6 of these novel therapeutic target genes were confirmed by semi-quantitative real time PCR in human adipocyte tissue: One of these genes is part of cell adhesion category (CD300LG), one encodes for protease inhibitors (SERPINB4), two are genes encoding for cell membrane specific receptors (BTNL9, ELTD1) and two for secreted proteins, respectively (PM20D1, PNLIPRP3). In further analyses we found distinct patterns of regulation for each gene in common human skin diseases. Knowledge of the full set of AAGs will not only lead to better understanding adipose tissue-related function and processes, but also open new avenues of research which could lead to better management and treatment options for adipose-associated diseases.

Health Services Research

P108

Patient benefits in the treatment of psoriasis: long-term outcomes in German routine care 2007–2014

M. A. Radtke, A. Langebruch, A. Jacobi and M. Augustin *University Medical Center of Hamburg-Eppendorf, Institute for Health Services Research in Dermatology and Nursing, 20246 Hamburg, Germany*

Background: Psoriasis is associated with significant patient burden. Few studies have specifically measured patient preferences and benefits.
Objectives: Outcomes assessment using the Patient Benefit Index (PBI) in nationwide psoriasis surveys comparing health care in 2014 and 2007.
Methods: Non-interventional, cross-sectional surveys conducted in 2007 and 2014 in randomly selected dermatological practices and clinics recording a) by physicians: previous treatments and comorbidity, clinical severity (PASI, BSA), b) by patients: quality of life (DLQI, EQ-5D) and patient-relevant therapeutic benefits (PBI).
Results: In 2014, a total of $n = 1265$ patients (43.4% female, mean age 52 ± 14.3 years.; mean disease duration 21.3 ± 15.2 years.) were included. Overall PBI was 2.8 ± 1.1. 91.6% of patients showed a more than minimum clinically relevant benefit (PBI >1). Patients treated with biologics showed the highest benefit (PBI 3.4 ± 0.8; 95%CI: 3.0–3.7) compared to patients with conventional systemic treatment (PBI 2.9 ± 1.0; 95%CI: 2.8–3.0) and patients treated with topical steroids (PBI 2.2 + 1.2; 95% CI: 1.9–2.4). Mean DLQI was 5.9 ± 5.9 and significantly lower in patients treated with biologics or conventional systemics (4.6 ± 5.2) compared to patients treated with topicals only (6.7 ± 5.5; $P < 0.0001$). In comparison with the 2007 survey ($n = 2009$), there was an increase of PBI from 2.5 ± 1.1 to 2.8 ± 1.1 and a gain of patients with high benefits by 30% (49.4% vs. 38.1%). DLQI decreased by 21.3% from 7.5 ± 6.4 to 5.9 ± 5.9 and the proportion of patients with DLQI >10 from 28.2% to 21.3%.
Conclusion: In German routine care, psoriasis patients have shown increased therapeutic benefits over time with highest benefits deriving from biologics.

Immunology

P109

L1-RG1 virus-like particle (VLP) vaccines directed against cutaneous human papillomaviruses (HPV)

B. Huber, C. Schellenbacher, C. Jindra, S. Shafti-Keramat and R. Kirnbauer *Laboratory of Viral Oncology (LVO), Division of Immunology, Allergy and Infectious Diseases (DIAID), Dermatology, Medical University of Vienna, 1090 Vienna, Austria*

Licensed multivalent HPV vaccines are comprised of major capsid protein L1-based VLP that provide type-restricted protection to the targeted genital HPV types and associated ano-genital disease. HPV types of genus beta (betaPV) are a distinct large group of cutaneous HPV that infect the skin soon after birth as element of a well-controlled commensal flora. BetaPV are hypothesized to play a role adjunct to the main carcinogen UV-light in the development of non-melanoma skin cancer (NMSC) in immunosuppressed patients. Common cutaneous types, most often HPV1/2/3/4/10/27/57 cause common and palmo-plantar warts, a significant burden for health care systems.
To develop a vaccine that targets cutaneous HPV, a cross-neutralization epitope of the minor capsid protein L2 (HPV16 L2 'RG1' homologue) of beta HPV17 or HPV4 was genetically inserted into the DE-surface loop of either HPV16, HPV5, or HPV1 L1, resulting in 16L1-17RG1, 5L1-17RG1, or 1L1-4RG1 chimeric fusion proteins. Proteins expressed by recombinant baculoviruses in Sf9 insect cells self-assembled into VLP verified by transmission electron microscopy. Following immunization of New Zealand White rabbits plus human-applicable alum-MPL adjuvant, immune sera were analyzed by ELISA and L1- and L2-based pseudovirion (PsV) neutralization assays. To fully evaluate cross-neutralization efficacy of L1-RG1 VLP vaccines, novel betaPV PsV were generated. Furthermore, efficacy of (cross-) protection was analyzed *in vivo* by a murine vaginal challenge model.
Specific RG1-peptide ELISAs indicated immunogenic RG1 epitope presentation by VLP, and immune sera revealed cross-neutralizing antibody titers from 25 to 1000 against HPV5/8/16/20/23/24/36/92/96 induced by 16L1-17RG1 VLP, against HPV5/20/24/36/92/96 by 5L1-17RG1 VLP, and HPV4 by the common cutaneous vaccine. Passive transfer of 16L1-17RG1 VLP-raised serum protected mice from genital challenge with tested HPV5/16/20/96 PsV but not HPV76, whereas 5L1-17RG1 VLP-raised serum provided cross-protection against HPV20 only. Further 1L1-4RG1 VLP-raised serum protected mice against challenge with HPV4 PsV.
In conclusion, chimeric L1-RG1 VLP vaccination targeting cutaneous HPV types is a promising strategy against common warts in children and the large skin cancer burden in immunosuppressed organ transplant recipients.

P110

Free fatty acids boost the activation of monocyte derived dendritic cells

D. Herbert[1], K. Stelzner[1], A. Lorz[1], Y. Popkova[2], J. Schiller[2], M. Gericke[3], M. Blüher[4], J. C. Simon[1] and A. Saalbach[1] [1]*Hospital of Leipzig University, Department of Dermatology, Venerology and Allergology, 04103 Leipzig, Germany;* [2]*University Leipzig, Institute of Medical Physics and Biophysics, 04107 Leipzig, Germany;* [3]*Hospital of Leipzig University, Institute of Anatomy, 04103 Leipzig, Germany;* [4]*Medical Faculty of the Leipzig University, Department of Medicine, 04103 Leipzig, Germany*

Psoriasis is a chronic inflammatory skin disease accompanied by a disturbed proliferation/differentiation of keratinocytes and a massive skin inflammation. A positive correlation between severity of psoriasis and obesity has been observed but mechanistic links are poorly understood. In chronic obesity fat depots enlarges and production of free fatty acids (FFA) mainly palmitic acid (PA), oleic acid (OA) by adipocytes increases. Moreover, in the skin we find the special situation where stromal cells and immune cells are closely co-located with the subcutaneous fat tissue. Here, we asked whether FFA might link obesity and severity of psoriatic skin inflammation.

In a cohort of 161 patients fasting FFA significantly correlated with the percentage of body fat. Analysis of patient subgroups according their glucose tolerance revealed that both gain of body fat and impaired glucose metabolism are two independent risk factors for an increase of fasting FFA in serum. Using a mouse model of high fat diet induced obesity showed that psoriasis-like skin inflammation was strengthened in obese mice compared to lean mice. In parallel, expression of TNFa, IL-1b, IL-6, IL-23 and cox-2 was augmented in obese mice.

Stimulation of human monocyte-derived DC and dermal fibroblasts with PA and OA indicated that FFA modulate immune responses by acting directly on DC functions and by interfering with the crosstalk between DC and fibroblasts. Pre-incubation of DC with PA or OA sensitizes DC resulting in an enhanced secretion of IL-23, IL-12, IL-6 and IL-1b upon stimulation. Moreover, PA induced PGE2 release from fibroblast that supported a TH1/TH17 immune response.

Our data showed that obesity facilitates psoriatic skin inflammation. FFA elevated in obesity might represent one link between obesity and severity of psoriasis.

P111 (O04/02)

Identification of a stable and migratory subset of tolerogenic IL-10 modulated human dendritic cells for optimized DC vaccination strategies

V. K. Raker[1], F. Kryczanowsky[1], E. Graulich[1], M. P. Domogalla[1] and K. Steinbrink[1] [1]*University Medical Center Mainz, Dermatology, 55131 Mainz, Germany*

Human IL-10 modulated tolerogenic dendritic cells (IL-10DC) are potent regulators of immunity by their ability to induce anergic regulatory $CD4^+$ T cells (iTregs). Within human IL-10 modulated tolerogenic dendritic cells we identified two subpopulations $CD83^{high}CCR7^{high}HLA\text{-}DR^{high}$ and $CD83^{low}CCR7^{neg}HLA\text{-}DR^{low}$IL-10DC. Compared to mature DC, $CD83^{low}$ IL-10DC showed diminished expression of costimulatory molecules and slight up-regulation of inhibitory molecules like ILT3, ILT4 and PD-L2 In contrast, $CD83^{high}$ IL-10DC revealed minor alterations in the expression of costimulatory molecules compared to mature DC but showed an increased expression of inhibitory molecules. However, both subsets of IL-10DC irrespective of their grade of maturation were potent inducers of iTreg. However, we found that that regulatory $CD4^+$ T cells ($iTreg^+$) induced by $CD83^{high}$ IL-10DC exhibited a significantly higher suppressive capacity compared to $CD4^+$ regulatory T cells ($iTreg^-$) generated by $CD83^{low}$ IL-10DC. In line with these results, $iTreg^+$ revealed a higher degree of activation by means of proliferation and cytokine secretion when compared to $iTreg^-$. With the perspective in mind to use modulated DC for iTreg induction in clinical settings, the migratory capacity of DCs play an important role. We found that $CD83^{high}$ rather than $CD83^{low}$ IL-10DC exerted a stronger migratory capacity towards the secondary lymph node-related chemokine CCL21. In addition, $CD83^{high}$ IL-10DC exhibited a stable tolerogenic phenotype under pro-inflammatory conditions, a prerequisite for clinical use in patients with inflammatory disorders. Furthermore, $CD83^{high}$ IL-10DC expressed high levels of surface and soluble CD25 (sCD25). In this context, we addressed the role of sCD25 functionally and found that sCD25 secreted by $CD83^{high}$ IL-10DC did efficiently inhibited T cell activation. We conclude that the selective use of $CD83^{high}$ IL-10DC for tolerance induction *in vivo* may contribute to an improvement of DC vaccination strategies against allergic and autoimmune diseases and transplant rejections.

P112

Contribution of mast cell interleukin 1-beta to UV-B induced skin inflammation

A. Smorodchenko[1], J. Scheffel[1], K. Krause[1], H. Bonnekoh[1], L. Kraas[1,4], E. Latz[2,3] and M. Maurer[1] [1]*Charité-Universitätsmedizin Berlin, Department of Dermatology and Allergy, 10117 Berlin, Germany;* [2]*Institute of Innate Immunity, University Hospital, University of Bonn, 53127 Bonn, Germany;* [3]*University of Massachusetts Medical School, Department of Medicine, Worcester, MA, USA;* [4]*Otto-von-Guericke University Magdeburg, University Clinic of Dermatology and Venereology, Magdeburg, Germany*

Mast cells (MCs) express a large repertoire of innate immune signaling receptors, which can be activated by pathogen associated molecular patterns (PAMPs) derived from bacteria and viruses, as well as danger associated molecular patterns (DAMPs), which originate from the host. Inflammasomes are an important class of innate immunity sensors for such signals. Via Interleukin-1 beta (IL-1beta) they drive early inflammatory responses during bacterial infection or tissue damage by sensing endogenous molecules that are altered by cellular stress. Recent studies have highlighted the importance of MC inflammasome activation for the development of skin lesions in patients with autoinflammatory conditions. The importance of MC inflammasome activation beyond these conditions has not been studied so far. Therefore, we started to explore whether MCs and IL-1beta contribute to inflammatory response induced by UV irradiation. Intracellular FACS staining in LPS stimulated bone marrow derived mast cells (BMMCs) revealed an enhanced expression of pro-IL-1β in MCs. Moreover, IL-1beta ELISA analyses of supernatants of BMMCs treated with the NLRP3 inflammasome stimulator Nigericin showed high amounts of IL-1β release, demonstrating that inflammasome activation in BMMCs can result in the production and release of IL-1beta. When we used mouse skin explants and exposed them to a single dose of 500 mJ/cm2 of UV-B light, we detected enhanced IL-1beta release in the supernatant by ELISA. To investigate whether MCs contribute to the IL-1beta release, immunohistochemistry (IHC) was performed and analysed by confocal microscopy, and we found that IL-1β was located close to MCs. Together these data suggest that MCs can produce IL-1beta after inflammasome activation and that MC-derived IL-1beta may contribute to UVinduced skin inflammation.

P113

Treg/Th17 and $\gamma\delta$ T cell plasticity in inflamed skin is modulated by the PPARγ- axis in psoriasiform dermatitis and psoriasis

M. V. Gatzka, K. Singh, A. Hainzl, R. Pandy, I. Krikki, C. M. Kröger, M. Wlaschek and K. Scharffetter-Kochanek *Universitätsklinikum Ulm, Department of Dermatology and Allergic Diseases, Ulm*

The high efficacy of modern drugs targeting the IL-23/IL-17 axis impressively reflects the pivotal role of IL-17 in human inflammatory skin diseases. Based on immunofluorescent analyses of biobank material from human psoriasis patients and of inflamed skin from CD18hypo PL/J mice with psoriasiform dermatitis – both showing a predominance CD3+IL-17+ T cells and presence of Foxp3+IL-17+ double-positive T cells among IL-17 producing cells -, we performed in-depth global gene expression studies and functional analyses in T cells isolated from murine CD18hypo PL/J psoriasiform skin to uncover potential novel mediators of Th cell plasticity in skin inflammation. In addition, we investigated the effects of fumaric acid esters (dimethylfumarate, DMF), a treatment option for psoriasis patients, on gene expression profiles of skin T cells in the CD18hypo PL/J psoriasis model.

At first, composition of the skin infiltrate of psoriasis biobank samples with significant numbers of $\gamma\delta$TCR+IL-17?γ, as well as Foxp3+IL-17+, and Foxp3+RORγt+ doublepositive cells among the strongly increased CD3 + IL-17+ fractions V indicative of potential conversion of regulatory T cells into Th17 cells V was quantified and evaluated in context with the severity and form of psoriasis. In global gene expression analyses of CD90.1+ T cells isolated from inflamed vs. healthy CD18hypo PL/J skin, we then identified the Peroxisome proliferator-activated receptor gamma (PPARγ) V previously implicated in negative regulation of Th17 differentiation in other autoimmune models V and co-regulators of PPARγ to be significantly down-regulated during skin inflammation. Whereas mRNA expression levels of redox-modulating enzymes, including Glutathion-peroxidases (Gpx4, -8, -10) and superoxidedismutases (SOD1, SOD3) responded well to treatment with DMF, other regulators such as Hif-1α, Nrf2 and Hemoxygenase (HO1) were not significantly altered in T cells after 14 days of DMF treatment. The functional role of the PPARγ axis in Treg/Th17 and $\gamma\delta$T cell plasticity was further evaluated in lentiviral overexpression studies in murine T cells, and in knockouse mouse models and confirmed in human skin samples at the expression level. Furthermore, binding of PPARγ co-regulators to the RORc promoter was detectable in chromatin immunoprecipitation (ChIP) assays, substantiating the potential role of these transcriptional co-regulators in Th17 differentiation.

In conclusion, T cell plasticity and the effect of DMF on the Foxp3/RORγt balance in skin $\alpha\beta$ and $\gamma\delta$ T cells *in vivo* and *in vitro* are at least in part dependent on the PPARγ-axis in the CD18hypo PL/J psoriasis model. Our data suggest that PPARγ and its co-regulators are down-regulated in skin T cells in an inflammatory milieu and maybe required to stabilize regulatory properties of T cells – and potentially other immune cells. This newly identified modulation of skin T cell plasticity and mode of action of redox-modulators such as DMF action is interesting for therapy of IL-17 mediated inflammatory diseases such as psoriasis and multiple sclerosis and provides novel insights into the molecular mechanisms of inflammation. In the future, targeted *in vivo* Treg reprogramming approaches could be applied to re-convert an aberrant immune system in autoimmune conditions of the skin and other organs.

P114

BAFF drives MC differentiation from CD34+ progenitor cells

P. Valentin, O. Schmetzer and M. Maurer *Charite, Department of Dermatology and Allergy, 10117 Berlin, Germany*

The differentiation of human mast cells (MCs) from progenitor cells requires a complex interplay of various cytokines including IL3 and SCF. B cell activating factor (BAFF), a cytokine of the TNF ligand family, importantly regulates B cell proliferation and differentiation, in part via BAFF receptor (BAFF-R). Recently, we found that about 25% of CD34+ stem cells express BAFF-receptor after 3 days of culture with IL3 and SCF. To test if BAFF can promote the development of peripheral CD34+ stem cell-derived mast cells (PSCMCs) we analyzed developing PSCMCs by flow cytometry, immunohistochemistry, and immunofluorescence for FcRI, CD117, and tryptase and -hexosaminidase content. BAFF led to a marked increase in FcRI and CD117 expression. By immunofluorescence, nearly 50% of PSCMCs (70 out of 144 cells) were double positive for CD117 and FcRI after the addition of 100 ng/ml BAFF and three weeks of culture, as compared to 20% of vehicle treated PSCMCs. Also, the addition of BAFF (titrated from 10–100 ng/ml) increased total cellular tryptase ($P = 0.033$) and -hexosaminidase ($P = 2 \times 10^{-7}$) content dose dependently and up to 2-fold as compared to vehicle treatment. These results indicate that BAFF may add to the effects of IL-3 and SCF on the development of PSCMCs from CD34+ stem cells.

P115

Pollen as modulator of the skin barrier and immune function

D. C. Dittlein[1,2], S. Gilles-Stein[1,2], I. Beck[1,2], J. Hiller[1], J. Durner[3], U. Frank[2,3], O. Groß[4] and C. Traidl-Hoffmann[1,2] [1]*Technische Universität München, Institute of environmental medicine, 86156 Munich, Germany;* [2]*CK-CARE, 7270 Davos, Switzerland;* [3]*HelmholtzZentrum München, Institute of biochemical plant pathology, 85764 Munich, Germany;* [4]*Technische Universität München, 81675 Munich, Germany*

Pollen extracts are a complex mixture of proteins, lipids, carbohydrates and other molecules which are by far not all identified or investigated in their effects on human cells. In addition, previous studies mostly concentrated on pollen derived allergens but only few studies have chosen an integrated approach comprising the effects pollen factors can have in their entirety on the skin epithelium. Therefore, a clear and general picture of the immune modulatory potential of pollen extracts is still missing. To gain deeper insight in this field we investigated in the current study not only the influence of pollen derived mediators on the morphology of human primary keratinocytes but analyzed also the effect on protein and mRNA expression including the impact on the inflammasome system.

For this purpose human primary keratinocytes were stimulated for different time points and in different concentrations with extracts of following plant pollen species: Ambrosia artemisiifolia, Phleum pratense, Betula pendula and Pinus sylvestris. Effects of the aqueous pollen extracts (APEs) on the morphology were checked by microscope and the impact on cytokine release and mRNA expression by ELISA and quantitative RT-PCR, respectively.

Besides concentration and specie depending effects on the cell morphology, results revealed an inducing effect on the release of pro-inflammatory cytokines. This included also IL-1β and IL-18, the hallmark products of inflammasome activation. Especially A. artemisiifolia extract was effective in the induction of pro-inflammatory cytokines and mostly exceeded the effects of the other pollen species. In Western Blot experiments inflammasome activation could be confirmed by showing activation of caspase-1. Strikingly the impact of APE on inflammasome mechanisms were enhanced when combined with UV-B as a second environmental factor. Furthermore, mRNA analysis showed impact of APE on the expression of Filaggrin, TSLP and PAR-2.

Taken together the current study provides new knowledge with the potential to be essential for the understanding of several mechanisms in the skin in response to plant pollen exposure. In addition, our results support the hypothesis that pollen influence the immunological barrier of the skin by triggering the inflammasome of keratinocytes per se and aggravating the effects of UV-B irradiation.

P116

Initiation of anti-tumor immune responses by repolarisation of tumor associated macrophages using innovative nanoparticles as siRNA carriers

J. Schupp[1], F. Förster[2], D. Bamberger[3], P. R. Wich[3], D. Schuppan[2] and A. Tüttenberg[1] [1]*University Medical Center of the Johannes Gutenberg University, Department of Dermatology, Mainz, Germany;* [2]*University Medical Center of the Johannes Gutenberg University, Institute of Translational Immunology, Mainz, Germany;* [3]*Johannes Gutenberg University, Institute of Pharmacy and Biochemistry, Mainz, Germany*

Immune evasion strategies enable tumor cells despite their immunogenicity to avoid immune surveillance by creating a highly suppressive tumor microenvironment. The immune suppression is mediated by cell-cell contact and secreted factors such as chemokines and cytokines. Tumor associated macrophages (TAM), also known as M2-polarized or alternatively activated macrophages, are major

players of the tumor microenvironment and have been shown to promote tumor growth by inducing neoangiogenesis, supporting metastasis and rendering tumor infiltrating lymphocytes (TIL) suppressive or apoptotic. TAM differentiate from tissue macrophages or blood derived monocytes after exposure to IL-4 and/ or IL-13. By disrupting the signal pathways responsible for TAM phenotype via siRNA mediated gene knockdown targeting receptors (IL-4R & CSFR1) and/or downstream transcription factors (STAT6, IRF4 & NOR1), we try to reprogram TAM to classical activated immunostimulatory M1 macrophages. To avoid degradation and unspecific cell uptake siRNA is bound to or encapsulated in nano-sized carriers provided by the SFB 1066 which has been established in Mainz and is a joint effort to develop nanoparticles as tumor therapeutic agents. Targeting of TAM can be achieved actively using antibodies against specific surface markers of this subset and mannose as ligand for CD206 or passively by exploiting the enhanced permeability and retention effect in tumor vessels and the high phagocytic activity of macrophages.

To screen potential siRNA targets for their ability to repolarize M2 macrophages and identify nanoparticles with high transfection rates, we have established an *in vitro* culture of human M1 (LPS and IFN-γ) and M2 (IL-4) macrophages derived from monocytes isolated from human PBMC. After verification of transfection and subsequent repolarization in our *in vitro* system, the siRNA loaded nanoparticles will be employed as therapy *in vivo* in a humanized mouse melanoma model. Acid degradable cationic dextran particles, which are able to efficiently encapsulate siRNA and have a size range of 100–150 nm, already proved to be a promising candidate because of low toxicity and high uptake rates in monocytes and macrophages without influencing the phenotype. In wild-type mice, nanoparticles accumulated preferentially in the liver where they showed high uptake rates in liver macrophages (70–80%). Following repeated treatments no toxicity could be detected in serum parameters. All things considered the use of engineered dextran nanoparticles as drug delivery systems targeting TAMs promises enormous potential to modulate immune tolerance towards tumors.

P117

The dual RAR and RXR agonist, alitretinoin, modifies leukocyte recruitment pathways and suppresses dendritic cell functions *in vitro* and *in vivo*

A. Kislat, S. Meller, B. A. Buhren, H. C. Friedrich and B. Homey *Heinrich-Heine University Hospital, Dermatology, 40225 Düsseldorf, Germany*

Retinoids regulate diverse cellular processes including proliferation, differentiation and moreover, the regulation and develpoment of the immune system. Clinical efficiancy have been proven in a variety of diseases, including acne vulgaris, pustular psoriasis, ichthyosis. The two nuclear receptors, the retinoic acid receptor (RAR) and the retinoic X receptor (RXR), mediate the effects of retinoids. Alitretinoin, binding to both RAR and RXR, demonstrated significant efficacy in the treatment of chronic hand eczema.

To gain inside the mode of action of alitretinoin *in vitro* and *in vivo* we analyzed effects of alitretinoin on patiens with chronic hand eczema. Further, we characterized the impact of alitretinoin treatment on keratinocytes as well as leukocyte subsets.

In vitro, alitretinoin alters chemokine expression of keratinocytes compared to acitretin, a RAR-agonist. Additional, alitretinoin inhibits the maturation of dendritic cells significantly higher in comparison to acitretin, leading to a higher impaired T cell activating capability.

In vivo, alitretinoin changes the expression patterns of cytokines and chemokines in the skin and the serum of patients. Further, 'skin-homing' effector T cells are decreased in the periphery. In addition, alitretinoin significantly decreased the proliferation of leukocytes following allogenic stimulation compared to proliferation before treatment.

In conclusion, alitretinoin is significantly more capable to alter the innate and adaptive immune responses by suppression of chemokine-induced leukocyte recruitment and inhibition of dendritic cell-mediated T cell activation.

P118

Reduced skin blistering in experimental epidermolysis bullosa acquisita after anti-TNF treatment

K. Bieber[1], M. Hirose[1], A. Kasprick[1], U. Samavedam[1], J. E. Klöpper[1], K. Kalies[3], D. Zillikens[2,1] and R. J. Ludwig[1] *[1]University of Luebeck, Luebeck, Germany; [2]University of Luebeck, Department of Dermatology, Allergology and Venereology, Luebeck, Germany; [3]University of Luebeck, Institute of Anatomy, Luebeck, Germany*

Epidermolysis bullosa acquisita (EBA) is a difficult-to-treat subepidermal autoimmune blistering skin disease (AIBD) with circulating and tissue-bound anti-type VII collagen antibodies. Different reports have indicated an increased concentration of tumor necrosis factor alpha (TNF) in the serum and blister fluid of patients with subepidermal AIBDs. Furthermore, successful anti-TNF treatment has been reported for individual patients with AIBDs. Here, we show that in mice, induction of experimental EBA by repeated injections of rabbit-anti mouse type VII collagen antibodies led to increased expression of TNF in skin, as determined by real-time PCR and immunohistochemistry. To investigate if the increased TNF expression is of functional relevance in experimental EBA, we inhibited TNF function using the soluble TNF receptor fusion protein etanercept (Enbrel®) or a monoclonal antibody to murine TNF. Interestingly, mice receiving either of these two treatments showed significantly milder disease progression than controls. In addition, immunohistochemical staining demonstrated reduced numbers of macrophages in lesional skin in mice treated with TNF inhibitors compared to controls. Furthermore, etanercept treatment significantly reduced the disease progression in immunizationinduced EBA. In conclusion, the increased expression of TNF in experimental EBA is of functional relevance, as both the prophylactic blockade of TNF and the therapeutic use of etanercept impaired the induction and progression of experimental EBA. Thus, TNF is likely to serve as a new therapeutic target for EBA and AIBDs with a similar pathogenesis.

P119

Analysis of myeloid cell populations and fibrosis in bleomycin- and HOClinduced scleroderma

J. Haub[1], V. K. Raker[1], Y. O. Kim[2], N. Lorenz[1], D. Schuppan[2] and K. Steinbrink[1] *[1]University Medical Center of the Johannes Gutenberg-University, Dermatology, 55131 Mainz, Germany; [2]University Medical Center of the Johannes Gutenberg-University, Translational Immunology, 55131 Mainz, Germany*

Systemic sclerosis (SSc) is a chronic autoimmune connective tissue disease which manifests in fibrosis, an accumulation of extracellular matrix (ECM) proteins in the skin and organs such as kidneys and lung. The pathogenesis of SSc is not fully understood yet, but early parameters encompass production of reaction oxygen species (ROS), vascular damage and a cellular infiltrate consisting of T cells and antigen-presenting cells (APC), like monocytes/macrophages and dendritic cells (DC). However, the role of APC in the early phase of the fibrosis has not been addressed so far. Therefore, in this study we have analyzed the function of myeloid cells and DC for scleroderma (Scl) development with the goal to identify novel targets for innovative therapeutic strategies. Mouse models resembling human cutaneous Scl can be induced by application of bleomycin or hypochlorous acid (HOCl). Here, we intradermally injected both agents over a period of four weeks. The development of skin thickness was monitored and skin punches were analyzed for fibrosis related parameters (qRT-PCR), extent of ECM accumulation (histology) and the inflammatory infiltrate (H&E, flow cytometry) at different time points during fibrosis development. Both models revealed a significant increase in dermal thickness and collagen deposition after four weeks, as hallmarks of Scl. However, clinical skin thickness, densely packed, sirius red stained collagen bundles and collagen crosslinks were more pronounced in HOCl-induced Scl. In parallel, there was a significant upregulation of procollagen α1(I) and α-SMA in HOCI animals, whereas IL-1β, MMP-13 and serum TGF-β1 levels were significantly increased in bleomycintreated mice. Flow cytometric analysis of the dermal infiltrate demonstrated an early cellular infiltrate containing mainly $CD19^+$ B cells, $CD4^+$ T cells, $CD11c^+$ DC and $CD11b^+$ myeloid cells, the latter one being significantly more prominent after HOCl injection. The percentage of $CD11c^+MHII^+$ representing DC and of $Ly6C^+MHCII^+$ and $F4/80^+MHCII^+$ monocytes/macrophages was elevated in HOCl-treated mice as well. Sub-analysis of $CD11b^+$ myeloid cells revealed that Scl mice exhibited a significant increase of inflammatory myeloid $CD11b^+Ly6C^{low-high}CD64^{low-high}$cells (HOCl>bleomycin). Especially in the HOCl model, activated dermal macrophages ($CCR2^{low}MHCII^{high}$) and monocyte-derived DC ($CCR2^{high}MHCII^{high}$) predominated over less activated $CD11b^+$ myeloid cells. Conclusively, the two models differ in certain aspects of scleroderma but in the HOCl-model, myeloid $CD11b^+MHCII^{high}$ cells highly correlate with fibrosis-related parameters. Therefore, analysis of both models is suggested to cover a broad spectrum of Scl-related symptoms. However, when studies aim to analyze early inflammatory processes, the HOCl-induced Scl-model should be considered in favor of bleomycin.

P120

Myeloid cell-restricted Insulin/IGF-1 signaling controls cutaneous inflammation

J. Knüver[1], S. Willenborg[1], X. Ding[1], M. D. Akyüz[1,2], C. M. Niessen[1,2], L. Partridge[4,5], J. C. Brüning[3,4] and S. A. Eming[1,4] *[1]University Hospital of Cologne, Dermatology, 50937 Cologne, Germany; [2]University of Cologne, Center for Molecular Medicine Cologne, 50937 Cologne, Germany; [3]Institute for Genetics, Department of Mouse Genetics and Metabolism, 50937 Cologne, Germany; [4]University of Cologne, Cologne Excellence Cluster on Cellular Stress Responses in Aging Associated Diseases, 50937 Cologne, Germany; [5]Max Planck Institute for Biology of Ageing, Cologne, Germany*

Myeloid cells are important regulators of tissue homeostasis and disease. Alterations in myeloid cell-restricted Insulin/IGF-1 signaling have recently shown to be of pivotal importance in the development of systemic inflammation and insulin resistance leading to diabetes. Pathological wound healing and inflammatory skin diseases are skin pathologies often associated with diabetes mellitus II, yet the responsible mechanisms are still unclear. Here we investigate whether myeloid cell-autonomous IR/IGF-1R signaling may be functionally linked with systemic insulin resistance and the development of skin inflammation. Therefore, we generated mice lacking both the Insulin and IGF-1 receptor on myeloid cells (IR/IGF-1RMKO). Whereas wound closure kinetics following acute skin injury was similar in control and IR/IGF-1RMKO mice, in two different conditions of dermatitis either induced by repetitive topical application of the detergent SDS or by high-dose UV radiation, IR/IGF-1RMKO mice were protected from inflammation, whereas controls developed severe skin dermatitis. Interestingly- although during the early phase in both inflammatory conditions the induction of epidermal pro-inflammatory cytokine expression was similar in control and IR/IGF-1RMKO mice-during the late stage epidermal cytokine expression was sustained in controls, however virtually abrogated in IR/IGF-1RMKO mice. This specific kinetic of epidermal cytokine expression was paralleled by proinflammatory macrophage activation in controls and a non-inflammatory phenotype in mutants. In summary, our findings provide novel insights for a pro-inflammatory IR/ IGF-1R-dependent pathway in myeloid cells that plays a crucial role in the dynamics of an epidermal-dermal crosstalk in cutaneous inflammatory reactions, and may add to the mechanistic understanding of diseases associated with pathological myeloid cell IR/IGF 1R signaling including diabetes mellitus II.

P121

Effect of Ras-Raf-Pathway inhibitors on the immune-phenotype of dendritic cells during melanoma therapy

F. K. Krebs, E. Hajek, S. A. Hahn, S. Grabbe, M. Bros and A. Tüttenberg *University Medical Center Mainz, Dermatology, 55131 Mainz, Germany*

Dendritic cells (DC) are major players of the adaptive immune system presenting high capability to detect antigens, including sensing and processing cancer cells. Empirical data of Vemurafenib treatment in late stages of melanoma show in a significant part of patients a relapse after initial response during long-term treatment. Though cytotoxic effects of Vemurafenib on melanoma cells have been thoroughly analyzed, little is known about the effect of Vemurafenib on the immunogenicity of immune cells, especially DC.

In the present study we investigated phenotypical and functional changes of human and murine DC during Vemurafenib treatment *in vitro*.

Human monocyte-derived DC were isolated from human PBMC and terminally differentiated by addition of inflammatory cytokines or LPS. Murine bone marrowderived DC (BMDC) were isolated and stimulated on day six with LPS to induce maturation. Different BRAF- inhibitors (Vemurafenib, Dabrafenib) and the MEK inhibitor Trametinib were added simultaneously to unstimulated and stimulated DC populations, and the phenotype and functions of resulting DC populations were analyzed.

In summary, we found Dabrafenib-dependent modulation of murine DC phenotypes: Unstimulated DC displayed a more mature surface marker phenotype after application of Dabrafenib. In contrast, when co-applied with a DC stimulus Dabrafenib attenuated DC activation. Interestingly, Vemurafenib and Dabrafenib induced elevated IL-1β production in murine DC at either state of activation. In addition, DC treated with Trametinib showed significantly impaired IL-10 production at stimulated state.

In human DC, we observed modulation of mature DC phenotypes resulting in a rather immature, tolerogenic phenotype under the influence of the different kinase inhibitors.

To conclude, our data show that different kinase inhibitors significantly influence the DC immune-phenotype. Our findings suggest that the cytostatic and anticancerogenic effects of these inhibitors affect not only cancer cells, but also DC and, thereby, may have a regulatory effect on immune response.

P122

Novel microparticles create a slow releasing depot for long-term immunostimulation in allergen-specific immunotherapy

M. Garbani[1], W. Xia[2], C. Rhyner[1], M. Prati[1], A. Scheynius[3], B. Malissen[4], H. Engqvist[2], M. Maurer[5], R. Crameri[1] and D. Terhorst[4,5] *[1]University of Zurich, Swiss Institute of Allergy and Asthma Research, Davos, Switzerland; [2]Uppsala University, Uppsala, Sweden; [3]Karolinska Institutet and University Hospital, Department of Medicine Solna, Translational Immunology Unit, Stockholm, Sweden; [4]Centre d'Immunologie de Marseille-Luminy, Marseille, France; [5]Charité University Medicine Berlin, Berlin, Germany*

Immunomodulatory interventions play a key role in the treatment of infections and cancer as well as allergic diseases. Adjuvants such as micro- and nanoparticles are often added to immunomodulatory therapies to enhance the triggered immune response. Here, we report the immunological assessment of novel and economically manufactured microparticle adjuvants, namely homogeneous strontium-doped hydroxyapatite porous spheres (SHAS), which we suggest for the use as adjuvant and carrier in allergen-specific immunotherapy.

Scanning electron microscopy revealed that the synthesis procedure developed for the production of SHAS results in a highly homogeneous population of spheres. *In vitro*, the release dynamics for the model antigen ovalbumin (OVA) bound to SHAS (SHAS-OVA) showed a first release burst within 2 h followed by a sustained release over the investigated time span of 20 h. Furthermore, SHAS-OVA did not have any necrotic or apoptotic effects on human monocyte-derived dendritic cells even at high densities.

In a murine model of allergen-specific immunotherapy for allergic asthmatic inflammation we found that ovalbumin released from subcutaneously injected SHASOVA was detectable several days longer in the draining lymph node in comparison with soluble OVA. Moreover, we identified tolerogenic CD11b+ migratory dendritic cells as the major subset of antigen presenting cells responsible for the presentation of OVA epitopes in the lymph node leading to sustained stimulation of both CD4+ and CD8+ T-cells. Allergen-specific immunotherapy with SHAS-OVA as compared to soluble OVA resulted in comparable humoral responses and higher efficacy as assessed by symptom scoring.
We conclude that SHAS may constitute a suitable carrier and adjuvant for allergenspecific immunotherapy with great potential due to its unique protein-binding properties.

P123 (O03/02)

Terminally differentiated human dermal fibroblasts are equal to bone marrow multipotent mesenchymal stromal cells in regulating macrophage differentiation and activity *in vitro* and in reducing inflammation in an *in vivo* peritonitis model

R. A. Ferrer[1,2], M. Grünwedel[1], I. Forstreuter[1,2], J. C. Simon[1,2] and S. Franz[1,2] *[1]University Leipzig, Department of Dermatology, Venerology and Allergology, 04103 Leipzig, Germany; [2]Transregio Collaborative Research Centre TRR67, Leipzig / Dresden, Germany*

It has been hypothesized that cells of mesenchymal lineage in the skin with progenitor status can reduce inflammation and induce a pro-repair M2 macrophage phenotype. These cells are phenotypically similar to dermal fibroblasts (dFb) but present differentiation potential, higher clonality and exert immunomodulation exclusively. Here we demonstrate that regardless of progenitor status, terminally differentiated human dFb are as effective as the prototypical immunoregulatory cells, bone marrow-derived multipotent mesenchymal stromal cells (BM-MSC), in inducing an M2 polarization of macrophages and reducing inflammatory activity of these cells in an pro-inflammatory context. Differentiation of human peripheral blood derived CD14+ monocytes to macrophages in co-cultures with BM-MSC or dFb resulted in macrophages with reduced release of inflammatory TNF and IL-12p40, abundant secretion of pro-resolution IL-10 and increased expression of the cell surface marker CD163, suggesting a M2 polarization although they have been stimulated with a M1 polarizing cytokine (GM-CSF). Mechanistically, dFb become activated by the inflammatory environment (GM-CSF or IL-1b/TNF) and respond with increased gene expression of the immunomodulatory mediators COX2 and TSG6 and consequent release of PGE2 and TSG6 protein. Both mediators are key for the immunomodulatory effect of dFb on macrophages, as shown by a similar shift of monocyte differentiation towards M2 when only supernatants of inflammatory activated dFb were used for differentiation experiments. Furthermore, siRNA silencing of any of these genes was sufficient to abrogate the described immunomodulatory effects. In a model of peritoneal inflammation induced by thioglycollate injection, C57BL/6 mice treated with either activated BM-MSC or activated dFb presented drastically reduced immune cell infiltration with lower concentrations of the inflammatory cytokines IL-1b and IL-12 in the peritoneal fluid demonstrating equal potent anti-inflammatory activity of BM-MSC and dFb in complex inflammatory processes *in vivo*. Macrophages obtained from the peritoneal lavage of these animals presented a reduction in the ratio of CD86/ CD206 expression when treated with either MSC or dFb, speaking in favor of a M2 differentiation *in vivo*. Our results show that non progenitor human dFb are effective regulators of macrophage polarization and inflammation suggesting that the hierarchy of the cells in the mesenchymal lineages of the skin does not segregate immunomodulatory capacity and that dFb might perform crucial immunoregulatory functions in the skin that remain to be investigated.

P124 (O03/06)

The alteration of the immunological landscape in a spontaneous melanoma mouse model

D. G. Mairhofer[1], A. Prokopi[1], C. H. Tripp[1], V. Flacher[2], K. Komenda[1], B. E. Clausen[3], S. Chen[4] and P. Stoitzner[1] *[1]Medical University of Innsbruck, Department of Dermatology, Venereology and Allergology, Innsbruck, Austria; [2]Institute de Biologie Moléculaire et Cellulaire, Laboratory of Immunopathology and Therapeutic Chemistry, Strasbourg, France; [3]University Medical Center of the Johannes Gutenberg-University Mainz, Institute for Molecular Medicine, Mainz, Germany; [4]Rutgers University, Susan Lehman Cullman Laboratory for Cancer Research, Piscataway, New Jersey, USA*

Mouse tumor models mimicking the human disease are valuable tools to gain insights into tumor immunity. The transgenic (tg) mouse model tg(Grm1)EPv is based on the overexpression of the metabotropic glutamate receptor 1 (Grm1), causing spontaneous melanoma development with 100% penetrance. Relevance of this model is given by the fact that around 60% of human melanoma samples are characterized by the same abberant expression of Grm1. Although the tg(Grm1)EPv mouse was used in several studies, nothing was known on the alterations in the immune system during melanoma development. In this study we characterized innate and adaptive immune cells in the tumor microenvironment and the draining lymph node to understand the immune evasion mechanisms in this spontaneous mlanoma mouse model.
Tumor growth was accompanied by a reduction of CD4+T cells including regulatory T cells in the CD45+leukocyte pool present in tumor tissue and draining lymph nodes. The percentages of CD8+T cells were unchanged, and these cells displayed an activated phenotype in tumor-bearing mice. However, CD8+T cells were unresponsive to ex vivo restimulation with gp100 peptide. In line with this, myeloidderived suppressor cells were recruited to the tumor tissue and produced Arginase-1, iNOS and TGF-beta to suppress CD8+T cell responses. In addition, we analysed the network of skin dendritic cells (DC) and their functional capabilities. With sorting and depletion experiments we detected that skin DC are able to cross-present the tumorassociated antigen gp100 to CD8+T cell responses. However, melanoma growth disturbed the DC network and especially Langerin + dermal DC were reduced and functionally impaired.
In summary, we observed that tumors are characterized by an immunosuppressive microenvironment and a dysfunctional DC network allowing these tumors to grow progressively.

P125

Targeting neutrophils in blistering skin diseases- reuse of old drugs

S. Ghorbanalipoor[1], U. Samavedam[2], K. Kalies[3], D. Zillikens[2] and R. J. Ludwig[1] *[1]University of Luebeck, 23562 Luebeck, Germany; [2]University of Luebeck, Department of Dermatology, Allergy, and Venereology, 23562 Luebeck, Germany; [3]University of Luebeck, Institute for Anatomy, 23562 Luebeck, Germany*

Objective: Besides playing a key role in innate immunity, increasing evidence indicates that neutrophils are also important in the molecular pathogenesis of various autoimmune diseases including autoimmune blistering skin diseases (AIBD). Due to a constantly ageing society, over the past 10 years, the incidence of AIBD has doubled in Germany while no treatment guidelines exist to date. Considering these facts, there is a strong demand for finding new therapeutic options with fewer side effects than systematically applied drugs. The current study aims to repurpose marketed drugs for new indications in treatment of AIBD. The philosophy of drug repurposing is underpinned by the emerging realization that common molecular pathways are often shared among seemingly diverse diseases. Therefore, drugs originally identified as efficacious in one disease could potentially be of therapeutic benefit in another disorder with lower costs, shorter development times and higher success rates.
Methods: The Prestwick Chemical Library (PCL), containing 1200 approved drugs, was screened using luminol-enhanced chemiluminescence reactive oxygen species (ROS) release assay. For complementary screening, dose dependent neutrophilinhibitory effect of the promising drugs was examined from 1 μM to 0.01 μM and an acellular ROS release assay was likewise performed with the corresponding doses. Next, they were tested in term of cytotoxicity using FACS analysis and subsequently, their effects on functional properties of neutrophils were evaluated using FACS analysis. To translate our *in vitro* results into an *in vivo* model, antibody-induced transfer model of Epidermolysis bullosa acquisita (EBA)- a model of neutrophil dominated AIBD- was used. Thereafter, skin samples were processed for histological and immunofluorescence analysis.
Results: Primary screening revealed that 33 (2.75%) screened drugs had significant effect on neutrophil ROS generation/activation by more than 50%. Analysis of the therapeutic groups represented by the 33 hits indicated that anti-bacterials (n = 14, 42%), Neuro(psycho)logicals (n = 7, 21%) were the most represented drug classes. Via the secondary screening, 6 drugs were identified which were further analysed in antibody-induced transfer EBA mouse model with physiologically relevant doses. 4 out of 6 drugs alleviated clinical disease severity in treated mice compared with corresponding vehicle-treated group. *In vivo* confirmation enabled the ranking of hits, with respect to their significance on reduction of severity of disease symptoms in mice. Of note, as a proof-of-concept result we identified one of hits that had previously been used for treatment of rheumatoid arthritis. Furthermore, an *in vivo* dose-dependent confirmation of 2 drugs which have not previously been described in treatment of autoimmune diseases is currently underway.
Conclusion: Here we identified 2 drugs with neutrophil-inhibitory effects that had not previously been characterized as general treatment of autoimmune diseases. Using antibody-induced transfer EBA mouse model, we revealed that these drugs might have therapeutic use in the treatment of autoimmune blistering diseases. However, further work is required to evaluate the impact of these drugs in other models of AIBD and to elucidate the potential pathways that these drugs could interfer with.

P126 (O05/05)

Desmoglein 3 and bullous pemphigoid antigen 180 specific T cells in lichen planus exhibit a Th1/Th17 phenotype

T. Schmidt, R. Stein, I. Stulberg, V. Eubel, R. Eming and M. Hertl *Philipps-University Marburg, Department of Dermatology and Allergology, Marburg*

Lichen planus (LP) is a common chronic inflammatory disorder of skin and mucous membranes whose immune pathogenesis has been linked to CD8+ T cell-mediated cytotoxity against epidermal keratinocytes. The epidermal (auto)antigen(s) which trigger LP have not been identified even though single cases of LP have shown clinical features and autoantibody profiles of bullous pemphigoid (BP) and pemphigus vulgaris (PV). We here analysed the reactivity and cytokine profile of peripheral T lymphocytes from 30 LP patients and 18 healthy controls against the autoantigens of BP, bullous pemphigoid antigen 180 (BP180) and PV, desmoglein 3 (Dsg3) and desmoglein 1 (Dsg1), respectively, by ELISPOT analysis. Ex vivo stimulated T cells were monitored for the release of interferon-γ (IFNγ), interleukin-5 (IL-5) and interleukin-17a (IL-17a). In LP, there was a statistically significant increase of IFNγ- and IL-17a-secreting T cells reactive with the immunodominant NC16a domain but not with the COOH-terminus of BP180. Accordingly, several LP patients showed IFNγ-dominated T cell reactivity against Dsg3. Th1 responses against BP180 and Dsg3 were directly correlated with the number of IL-17a+ T cells indicative of an IFNγ/IL-17a T cell response. Of note, IL-5 secreting T cells reactive with BP180 and Dsg3 were not significantly elevated in LP. In contrast to the LP patients, PV patients (n = 6) and BP patients (n = 6) showed IL-5-dominated Th2 responses against Dsg3 and BP180, respectively. These findings show for the first time that LP is associated with a Th1/Th17 dominated T cellular response against BP180 and Dsg3, the autoantigens of BP and PV, two autoimmune disorders which are linked to a Th2-driven pathogenesis. Thus, the cytokine profile of autoreactive T cells which target specific autoantigens of the skin seems to be critical for the evolving clinical phenotype.

P127

Laser-assisted topical immunization with antigen-antibody complexes to target skin dendritic cells

C. H. Tripp[1], M. Lohmueller[1], J. Idoyaga[2] and P. Stoitzner[1] *[1]Medical University of Innsbruck, Department of Dermatology, Venereology and Allergology, 6020 Innsbruck, Austria; [2]Stanford University School of Medicine, Department of Microbiology and Immunology, 94305 Stanford, CA, USA*

Skin dendritic cells (DC) are very potent antigen presenting cells and the prime cells to induce immune responses against cutaneous infection and tumours. Antigen can be targeted to DC with the help of antibodies against surface molecules, such as the lectin receptors DEC-205 and Langerin. We know from preliminary results that antibody-antigen complexes penetrate poorly into barrier-disrupted skin as achieved by repeated tape stripping of skin. For improved delivery we tested laser poration with the infrared laser device from Pantec Biosolutions AG (P.L.E.A.S.E.®) that generates aqueous micro-pores of defined depth in the skin. Through these newly formed pores it should be possible to deliver larger molecules such as antibody-antigen conjugates for immunization. We immunized mice with DEC-205-OVA or Langerin-OVA through laser-treated ear skin in comparison to intradermal application and measured activation of antigen-specific CD8+T cells a week later. After intradermal immunization with DEC-205-OVA we detected higher numbers of pentamer+ CD8+ T cells than with a control antibody conjugated to OVA. This correlated with enhanced cytotoxic T cell responses *in vivo*. Langerin- OVA immunization was less effective as we have shown earlier. When we checked the penetration and transport of fluorescence-conjugated anti-Langerin and anti- DEC-205 antibodies after laser pretreatment of skin we observed that antigenantibody complexes are not taken up by skin DC. We are currently optimizing the settings for laser poration to investigate the full potential of this immunization approach.

P128

Parkinson's disease and multiple sclerosis are not associated with a higher incidence of autoantibodies against structural proteins of the dermal-epidermal junction

A. Recke[1,2], A. Oei[1], F. Hübner[2], K. Fechner[3], J. Graf[4], J. M. Hagenah[4], C. May[5], D. Woitalla[6], A. Salmen[7], D. Zillikens[2], R. Gold[7], W. Schlumberger[3] and E. Schmidt[1,2] *[1]University of Luebeck, 23538 Luebeck, Germany; [2]University of Luebeck, Department of Dermatology, 23538 Luebeck, Germany; [3]Euroimmun AG, Institute of Experimental Immunology, 23560 Luebeck, Germany; [4]University of Luebeck, Department of Neurology, 23538 Luebeck, Germany; [5]Ruhr-University Bochum, Medizinisches Proteom-Center, Bochum, Germany; [6]Katholische Kliniken Ruhrhalbinsel gGmbH, Department of Neurology, Essen, Germany; [7]Ruhr-University Bochum, Department of Neurology, 44791 Bochum, Germany*

Bullous pemphigoid (BP), the most frequent autoimmune blistering disease, is characterized by autoantibodies against two proteins of the dermal-epidermal junction, BP180 (type XVII collagen), and BP230 (bullous pemphigoid antigen 1, BPAG1). Two peculiar clinical features of BP are the old age with a mean age at disease onset between 75 and 80 years and the association with neurological diseases. Neurological diseases can be diagnosed in a considerable proportion of BP patients, including cognitive impairment, stroke, epilepsy, and, most strinkingly, Parkinson's disease (PD) and multiple sclerosis (MS). Vice versa, patients with MS are more likely to develop BP. In the present study, we addressed the hypothesis that the autoimmune reaction against BP180 and BP230 is triggered by the inflammatory or degenerative processes in the CNS. According to this hypothesis we expected to detect serum autoantibodies against these target antigens in a higher frequency in patients with PD

and MS. We compared three age- and sexmatched groups of patients with PD ($n = 75$), other neurological diseases ($n = 75$) and a healthy controls ($n = 75$). Furthermore, we prospectively collected sera from another PD cohort ($n = 50$), patients with non-inflammatory skin diseases older than 75 years ($n = 65$), and patients with MS ($n = 50$). Based upon former studies that described a positive reactivity against BP180 and BP230 in about 1–2% of healthy individuals, we estimated a power of 0.51–0.86 to detect a clinically relevant 5-fold increase in incidence of autoreactivity in cohorts with neurologic diseases, compared to healthy controls. Reactivity against BP180 and BP230 in all sera was detected with a panel of diagnostic assays comprising of (i) indirect IF microscopy on a BIOCHIP® mosaic (monkey esophagus, split human skin, recombinant BP180 NC16A, HEK293 expressing the BP180 ectodomain, the BP230 globular domain, and full length BP230; Euroimmun, Luebeck, Germany), (ii) BP180 NC16A ELISA, (iii) BP230 ELISA (both Euroimmun), (iv) Western blotting with extracellular matrix of cultured human keratinocytes (for detection of laminin 332 and BP180), (v) indirect IF microscopy on monkey esophagus, and (vi) 1 M NaCl-split human skin (both inhouse tests). No significant differences were seen between the frequency of serum autoantibodies against proteins of the dermal-epidermal junction in patients with PD and MS compared to controls. In none of the samples, reactivity against BP180 or BP230 could be demonstrated by all test methods although the BP180NC16A ELISA was more often positive (7 of the total 390 samples) than the corresponding BIOCHIP mosaic® substrate (1 of total 390 samples). Altogether, antibodies against the dermal-epidermal junction were observed in 4 of 175 (2.3%, 95% CI 0.9–5.7%) of PD/ MS sera and 16 of 215 (7.4%, 95% CI 4.6–11.7%) of control sera in line with known specificities of 98–99% of the employed test systems. In summary, we did not find a clinically relevant increase of autoantibodies against BP180 and BP230 in patients with PD and MS, compared to controls. This indicates that despite the clear epidemiological association there is no extended clinically latent phase before the manifestation of BP in PD and MS patients.

P129 (O04/01)

Novel insights in the link between type 2 innate signals and initiation of profibrotic pathways

S. Willenborg[1], J. A. Knipper[1], J. Brinckmann[2], W. Bloch[3], T. Maaß[4], R. Wagener[4], T. Krieg[1], T. Sutherland[5], A. Munitz[6], M. E. Rothenberg[7], A. Niehoff[8], R. Richardson[9], M. Hammerschmidt[9], J. E. Allen[5] and S. A. Eming[1] *[1]University of Cologne, Department of Dermatology, 50937 Cologne; [2]University of Luebeck, Department of Dermatology, 23562 Luebeck; [3]German Sport University Cologne, Department of Molecular and Cellular Sport Medicine, 50933 Cologne; [4]University of Cologne, Department of Biochemistry, 50937 Cologne; [5]University of Edinburgh, Institute of Immunology and Infection Research, EH9 3FL Edinburgh, United Kingdom; [6]The Tel-Aviv University, Department of Clinical Microbiology and Immunology, 69978 Ramat Aviv, Israel; [7]University of Cincinnati College of Medicine, Division of Allergy and Immunology, OH 45229-3 Cincinnati, USA; [8]German Sport University Cologne, Institute of Biomechanics & Orthopedics, 50933 Cologne, Germany; [9]University of Cologne, Institute of Developmental Biology, 50674 Cologne, Germany*

Activation of the immune response is a critical early event during injury that determines the outcome of tissue restoration towards regeneration or replacement of the damaged tissue with a scar. The mechanisms by which immune signals control these fundamentally different regenerative pathways are largely unknown. In this study we have demonstrated that during skin repair in mice interleukin-4 receptor α (IL-4Rα)-dependent macrophage activation controlled collagen fibril assembly, and that this process was important for effective repair while having adverse profibrotic effects. We could show that in mice with myeloid cell-restricted IL-4Rα- deficiency (Il4rαMKO) skin repair was associated with delayed wound closure, massive hemorrhages in the granulation tissue, and disturbances in extracellular matrix architecture. Ultrastructural analysis of wound tissue in Il4rαMKO mice revealed an abnormal collagen fibril assembly. Intriguingly, HPLC-based analysis of the granulation tissue revealed an altered collagen cross-link pattern when compared to control mice. Whereas granulation tissue in control mice was characterized by dihydroxy lysinonorleucine (DHLNL) collagen cross-links, a typical feature of fibrotic tissue, these crosslinks were significantly reduced in Il4rαMKO mice.

To identify macrophage-derived mediators that control the formation of extracellular matrix architecture, we analyzed flow cytometry sorted wound macrophages. Interestingly, wound macrophages in Il4rαMKO mice revealed significantly reduced expression of Relm-α, a small cysteine-rich secreted molecule that is a hallmark of alternatively activated macrophages and has been associated with experimental fibrosis and pro-fibrotic conditions in human diseases. By using an *in vitro* macrophage-fibroblast co-culture system we identified Relm-α released from macrophages as inducer of lysyl hydroxylase 2 (LH2) expression in fibroblasts. LH2 is known to play a pivotal role directing DHLNL collagen cross-links.

To substantiate a direct role of Relm-α in skin repair we characterized the wound healing response in Relm-α deficient ($Retnla^{-/-}$) mice. Notably, we detected intriguing parallels regarding morphological, structural and biochemical alterations of the wound healing response in $Retnla^{-/-}$ and Il4rαMKO mice. As such the repair response in $Retnla^{-/-}$ mice was characterized by a highly hemorrhagic granulation tissue, transient delay in wound epithelialization, and altered assembly and size of collagen fibrils. Most importantly, HPLC-based analysis revealed reduced DHLNL collagen cross-links in $Retnla^{-/-}$ mice when compared to controls, and this was associated with reduced expression of LH2 in complete wound tissue. Importantly, local application of recombinant Relm-α restored LH2 expression and rescued disturbed granulation tissue formation in Il4rαMKO mice. Collectively, our findings provide novel mechanistic insights in the link between type 2 immunity and initiation of profibrotic pathways.

P130

Short chain fatty acids induce regulatory T cells by modulating dendritic cells.

A. Schwarz, A. Bruhs and T. Schwarz *University Clinics Schleswig-Holstein, Dermatology, 24105 Kiel, Germany*

We recently observed that commensal microbe-derived short chain fatty acids (SCFA) like sodium butyrate induce regulatory T cells (Treg) not only in the colon but in also in the skin. Thus, SCFA produced by commensal skin bacteria under anaerobic conditions may regulate immune responses in the skin via activation or induction of resident cutaneous T cells. Accordingly, application of sodium butyrate inhibited the induction of contact hypersensitivity (CHS). Since injection of sodium butyrate-treated and hapten-coupled bone marrow derived dendritic cells (BMDC) rendered recipient mice unresponsive to sensitization, we postulated that dendritic cells might play an important role in SCFA-mediated induction of Treg. Injection of lymph node cells and splenocytes obtained from mice which were injected with butyrate-treated BMDC suppressed the induction of CHS in the recipients, indicating that butyrate-treated BMDC induce Treg. To clarify whether these Treg express the Treg specific transcription factor Foxp3, we utilized DEREG mice (DEpletion of REGulatory T cells) in which Foxp3-positive cells can be depleted by the injection of diphtheria toxin (DT). Lymph node cells obtained from DEREG donors injected with butyrate-treated BMDC significantly suppressed CHS in the recipients. In contrast, sensitization was not suppressed upon transfer of cells obtained from DEREG mice which were treated with DT upon injection of butyrate – treated BMDC. This indicates that Treg induced by butyrate-treated BMDC express Foxp3. Moreover FACS analyses revealed a down-regulation of the expression of MHC class II (major histocompatibility complex class II) and of the costimulatory molecule B7-2 in butyrate-treated BMDC. In addition, butyrate induced the secretion of the immunosuppressive cytokine interleukin-10 by BMDC. Together these data imply that butyrate switches dendritic cells from a stimulatory into a regulatory phenotype which finally induces Treg.

P131

Systemic treatment with fumaric acid esters or TNF-alpha blockade normalizes the bacterial microbiota in cutaneous lesions of psoriasis patients

C. Baumann[1], V. Oji[1], A. Tsianakas[1], U. Dobrindt[2], T. Rosenbach[3], J. Kalinowski[4], T. A. Luger[1] and K. Loser[1] *[1]University of Muenster, Department of Dermatology, 48149 Muenster, Germany; [2]University of Muenster, Institute of Hygiene, 48149 Muenster, Germany; [3]Bredlich, Rosenbach and Thiele, 49078 Osnabrück, Germany; [4]University of Bielefeld, Center for Biotechnology, 33615 Bielefeld, Germany*

The skin forms a critical interface between the human body and the external environment, prevents water loss, and represents the first barrier towards pathogens. However, the skin also acts as an ecosystem providing physiologically and topographically distinct niches for microbial communities and it has been shown that commensal microbes influence the development and progression of various skin diseases. Psoriasis is a chronic, immune mediated inflammatory skin disease affecting more than 2% of the population. Although the pathomechanisms are still elusive the disease seems to result from a combination of genetic and environmental factors. Accordingly, putative loci for genetic susceptibility were identified on the basis of genome-wide linkage studies and moreover, various cutaneous microorganisms have been implicated in the pathogenesis of psoriasis. Actinobacteria or Staphylococcus epidermidis were detected in healthy skin and seem to protect humans from the invasion of pathogenic bacteria by secreting toxic anti-microbial peptides demonstrating that the skin and the cutaneous microbiota co-exist in a well-established balance and furthermore, suggesting that alterations in the composition of the cutaneous microbiota might promote the progression of skin diseases. Targeted amplicon sequencing of the 16S rRNA gene revealed an over-representation of the phyla Firmicutes or Proteobacterium as well as an underrepresentation of the phylum Actinobacterium in inflammatory skin from psoriasis patients as compared to non-lesional skin from the same individual suggesting that the cutaneous microbiota might affect the pathogenesis of psoriasis. Hence, we speculated that 'normalization' of the skin microflora in cutaneous lesions could be an important prerequisite for successful treatment and aimed at investigating if and how the cutaneous microflora changes in lesional skin from the same psoriasis patients before and at different time points after systemic treatment with fumaric acid esters (Fumaderm®) or TNF-alpha blockade. After having established the isolation of sufficient amounts of microbial DNA from swabs that were used to collect commensals from lesional and corresponding non-lesional skin areas of individuals with psoriasis, we amplified the variable regions V3–V4 of the 16S rRNA genes in a PCR reaction. Subsequently, barcoded libraries were prepared and subjected to high throughput next generation sequencing using the Illumina MiSeq® technology. As expected the percentage of proteobacteria was increased in lesional skin before treatment compared to non-lesional skin whereas the percentage of actinobacteria was reduced. However, after treatment with fumaric acid esters and after systemic TNF-alpha blockade (treatment with Enbrel®) we observed markedly decreased levels of proteobacteria and an increase of actinobacteria in lesional skin versus before treatment. Notably, psoriasis patients receiving systemic cyclosporine or methotrexate did not show a reduction in the relative abundance of proteobacteria or an up-regulation of actinobacteria after as compared to before treatment. Interestingly, the alterations in the microbial communities in lesional skin from patients treated with Fumaderm® or Enbrel® correlated with a reduced PASI score. Furthermore, systemic treatment with fumaric acid esters or TNFalpha blockade down-regulated the numbers of different phyla in psoriatic skin, thus suggesting that Fumaderm® and Enbrel® might 'normalize' the microbiota in cutaneous lesions.

P132

Local treatment with the Yersinia outer protein M (YopM) ameliorates ongoing psoriasis in mice

M. Schneeweiß[1], M. Poceva[2], A. Gossens[2], C. Rüter[2], T. A. Luger[1] and K. Loser[1] *[1]University of Muenster, Department of Dermatology, 48149 Muenster; [2]University of Muenster, Center for Molecular Biology of Inflammation, 48149 Muenster*

The Yersinia enterocolitica outer protein M (YopM) was recently identified as a novel cell-penetrating peptide, which autonomously enters eukaryotic cells, such as immune or epithelial cells, independent of translocation via the T3SS secretion system. After cell penetration YopM down-regulated the expression of pro-inflammatory cytokines like TNF-alpha or IFN gamma in natural killer cells and macrophages as determined by quantitative real-time PCR and thereby, is essential for the virulence of Y. enterocolitica by diminishing the host's immune response. In contrast to all other known Yops, YopM apparently does not possess enzymatic activity and was suggested to exert its anti-inflammatory effect by inhibiting caspase-1 activation. Hence, recombinant YopM (rYopM) might be of interest as a novel treatment option of inflammatory disorders and in particular the ability of YopM to autonomously enter cells and breach barriers suggests the cellpenetrating peptide as a promising target for the topical treatment of inflammatory skin diseases, like psoriasis. To investigate the anti-inflammatory potency of locally applied rYopM during established cutaneous inflammation *in vivo* we used the mouse model of imiquimod-induced psoriasis, which is characterized by the expansion of Th1 and Th17 cells as well as increased IL-23 levels in lesional skin. To elicit a psoriasis-like phenotype, mice were treated daily with 62.5 mg Aldara-cream (5% imiquimod) on the shaved back and after onset of disease mice were additionally treated locally with rYopM. Interestingly, subcutaneously injected rYopM significantly reduced ongoing skin inflammation as evidenced by the decreased acanthosis, parakeratotic hyperkeratosis and papillomatosis in treated mice compared to controls, which of course translated into a diminished clinical score and scratching behavior. Notably, in addition topical rYopM treatment was able to markedly reduce the levels of cytokines associated with psoriasis progression, such as IL-17 or IL-36 in lesional skin as quantified real-time PCR. However, the beneficial effect of rYopM was restricted to the treated skin area and did not elicit systemic effects since multicolor flow cytometric analysis of the skin-draining (inguinal) lymph nodes did not reveal any differences in numbers or phenotype of immune cell subsets known to contribute to psoriasis development. Next, we generated a rYopM-containing cream (Moppi-Y) to investigate whether the cell-penetrating peptide, even when applied as topical ointment instead of injected subcutaneously, might be able to efficiently inhibit the progression of ongoing psoriasis. Strikingly, mice that received imiquimod plus Moppi-Y showed a markedly reduced clinical score and scratching frequency as compared to imiquimod plus placebo-treated controls. Additionally, Moppi-Y downregulated the imiquimod-induced expression of pro-inflammatory cytokines, such as IL-17, in lesional skin. Importantly, even after long term topical treatment with rYopM (daily application for 8 weeks) we did not observe the induction of anti-YopM antibodies, hence suggesting rYopM as a promising protein for future analysis and for the local treatment of inflammatory skin diseases.

P133

IgE autoreactivity in bullous pemphigoid

P. Freire, P. Heil and G. Stingl *Medical University of Vienna, 1090 Vienna, Austria*

Bullous pemphigoid (BP) is an auto-immune disease typically associated with old age. It is characterized by bullae at the dermal-epidermal junction (DEJ) that are thought to be induced by the binding of auto-antibodies. These antibodies can recruit inflammatory cells through complement activation, culminating in the proteolytic destruction of cell adhesion structures. While IgG has been the class consistently associated with the disease, more recent studies point to a potential involvement of IgE. In line with previous literature, we have detected significantly higher levels of NC16a-specific IgE in the sera of BP patients comparing with healthy controls, via ELISA. Consistently, using whole skin lysates for immunoblotting, we have also demonstrated peripheral BP IgE reactivity against antigens with approximately 60, 120, 180 and 230 kD. These likely represent intra- and extra-cellular domains of BP180 and the full-length BP180 and BP230 proteins, respectively. Furthermore, we have

found IgE in perilesional skin of 21 out of 32 (66%) BP patients. This IgE was not found at the DEJ, but instead on the surface of mast cells and eosinophils, most likely bound as an immune complex. We have evidence that the high-affinity receptor for IgE is the primary molecule involved in this interaction and that eosinophils are expressing FcεRI in BP patients. Given that the clinical picture of BP consists of erythema and bullae, appearing alone or concomitantly, an association between self-reactive IgE and urticarial-like lesions is therefore plausible and suggests an alternative pathway of disease pathogenesis. Uncovering the dominant epitopes for both IgG and IgE in different presentations of the disease could further clarify this question and additionally argue for the development of new IgE-based therapeutic approaches.

P134 (O01/06)

ADAM17 is a psoriasis-relevant check-point controlling Th17-programming by inflammatory dermal dendritic cells

A. Kunze[1], S. Oehrl[1], P. Beckhove[2], G. Murphy[3], A. H. Enk[1] and K. Schäkel[1] [1]*University Hospital Heidelberg, Dermatology, 69120 Heidelberg, Germany;* [2]*German Cancer Research Center (DKFZ), Translational Immunology, 69120 Heidelberg, Germany;* [3]*University of Cambridge, Oncology, CB2 0RE Cambridge, United Kingdom*

Psoriasis is a chronic inflammatory skin disease in which activated 6-sulfo LacNAc expressing (slan) dendritic cells (slanDC) function as inflammatory dermal dendritic cells. slanDC have a high IL-23-, IL-12-, IL-1β- and TNF-α- producing capacity and thereby program Th17/Th1 dominated T cell responses. Recently, an imbalance of ADAM17-expression and its inhibitor TIMP3 was found in the epidermal compartment in psoriasis, and restoration of TIMP3 levels induced regression of skin lesions (Guinea-Viniegra et al., Sci Transl Med 2014). We here asked for the functional relevance of ADAM17 for inflammatory dermal DC in psoriasis. We demonstrate by immunofluorescent staining the upregulation of ADAM17 and the downregulation of TIMP3 for the dermis as well as the epidermis. slanDC but not CD1c+ DC or CD141+ DC were found to express cell surface ADAM17 as revealed by flow cytometry and the enzymatic activity of ADAM17 could be demonstrated by a specific fluorescence peptide assay. Addition of the endogenous protease inhibitor TIMP3 to slanDC inhibited ADAM17 activation, and most interestingly, it blocked LPS-induced IL-23- and IL-12-production by 60 to 70% – identical results were obtained with a highly specific ADAM17-blocking antibody D1A12. Asking for the biologic relevance of these findings in the context of psoriasis, we set up cocultures of CD4+ T cells from psoriasis patients stimulated with allogeneic slanDC in the presence of the specific ADAM17-blocking antibody D1A12. A restimulation of these cultures after 7 days revealed a largely reduced IL-17 production of T cells, being identical to the effects achieved with an IL-12/IL23p40-specific antibody. Taken together we identified ADAM17 to be involved in the control of Th17 responses is psoriasis.

P135

Exploring the role of $\gamma\delta$ T cells in human hair follicle immunopathology: Indications that Vδ1+ T cells are cytotoxic for 'stressed' hair follicles and may be involved in alopecia areata pathobiology

Y. Uchida[1,2], M. Bertolini[1], M. Alam[1], A. Gilhar[3], T. Kanekura[2], A. Rossi[4] and R. Paus[1,5] [1]*University of Muenster, Dermatology, Muenster, Germany;* [2]*Kagoshima University, Department of Dermatology, Kagoshima, Japan;* [3]*Technion-Israel Institute of Technology and Flieman Medical Center, Haifa, Israel;* [4]*University 'La Sapienza', Rome, Italy;* [5]*University of Manchester, Manchester, UK*

In murine skin, $\gamma\delta$ T cells play key roles in anti-infection defense, immunoregulation, tumor immunosurveillance, wound healing, and both hair follicle (HF) cycling and HF neogenesis. However, the role of $\gamma\delta$ T cells in the human HF remains completely unknown. Human $\gamma\delta$ T cells have been shown to have a dual role; they exert immunosuppressive as well as cytotoxic activities mediating stress surveillance through TCR, or non-TCR stress receptor (e.g. Natural killer group 2D-positive (NKG2D)) and exert various functions (e.g. anti-tumor) to maintain tissue integrity. In order to explore the role of $\gamma\delta$ T-cells in human HFs, we have first charactered their number, location, and subtype in the HF epithelium and perifollicular dermis during different hair cycle stages.

As expected, intrafollicular and the majority of perifollicullar $\gamma\delta$ T cells in healthy human skin were almost exclusively Vδ1+. Interestingly, In human scalp HFs, intrafollicular $\gamma\delta$ T cells were virtually absent in catagen HF epithelium and were restricted to the distal HF epithelium above the isthmus in anagen VI and, very rarely, in telogen HFs. Strikingly, $\gamma\delta$ T cells populated the perifollicular dermis only around anagen and catagen HFs. This raises the question whether Vδ1+ T cells are also actively involved in the control of human HF cycling.

NKG2D+ cells (incl. NK, NKT, and CD8+ T cells) play an important role in the pathogenesis of alopecia areata (AA), one of the most common human autoimmune diseases. Although human $\gamma\delta$ T cells also express NKG2D, their role in AA pathogenesis has not been explored yet. We therefore also investigated $\gamma\delta$ T cells in AA patients. In these, Vδ1 (but not Vδ2) TCR+ cells densely populated the perifollicular inflammatory cell infiltrate of lesional AA HFs and prominently infiltrated the hair bulb epithelium. Importantly, these $\gamma\delta$ TCR+ cells expressed NKG2D, the receptor for MICA and ULBP3, which are overexpressed in/around lesional AA HFs. Therefore, we also attempted to address the hypothesis whether NKG2D+ Vδ1 T cells recognize MICA-overexpressing, 'stressed' human HF epithelium and attack it in AA. To this end, we have co-cultured autologous skin-derived $\gamma\delta$ T cells with microdissection-'stressed' (day 1 after microdissection) and 'non-stressed' human scalp HF from healthy donors (day 4 after microdissection). Interestingly, when autologous $\gamma\delta$ T cells were co-cultured with 'stressed' HFs they induced substantial HF cytotoxicity (as assessed by histology and LDH release), up-regulated ectopic MHC class I expression in the HF epithelium, decreased the proliferation and increased apoptosis in the ORS and hair matrix of 'stressed' human HFs. None of these events was seen when autologous $\gamma\delta$ T cells were co-cultured with 'nonstressed' HFs.

These preliminary results are consistent with the hypothesis that NKG2D+ Vδ1 T cells may contribute to the cytotoxic damage imparted on MICA-overexpressing, 'stressed' human HF epithelium in AA. While Vδ2 T cells have been implicated in the pathogenesis of psoriasis, an involvement of NKG2D+ Vδ1 T cells in a bona fide, T cell-dependent human autoimmune disease, namely in human HF immunopathology and immune privilege collapse during AA, is an exciting novel pathobiology concept. Next, it needs to be investigated whether targeting NKG2D+ Vδ1 T cells impacts on the course of AA.

P136

Characterization of IL-21-producing T cells in pemphigus vulgaris

R. Pollmann[1], T. Schmidt[1], C. Moebs[1], M. Seipelt[2], B. Tackenberg[2], W. Pfuetzner[1], M. Hertl[1] and R. Eming[1] [1]*Philipps University Marburg, Department of Dermatology and Allergology, 35043 Marburg;* [2]*Philipps University Marburg, Department of Neurology, 35043 Marburg*

Pemphigus vulgaris (PV) is a potentially life-threatening autoimmune disease in which autoantibodies (auto-ab) against Desmoglein (Dsg) 3 and Dsg1 cause loss of keratinocyte adhesion resulting in painful blisters and erosions of the skin and mucous membranes. The auto-ab response in PV depends on different CD4+ T cell subsets producing a variety of cytokines that are crucial for the induction of the auto-ab response. However, the role of disease promoting cytokines in PV has yet not been fully characterized. Our work focuses on IL-21, a pleiotropic cytokine that promotes B cell proliferation and antibody production, that is predominantly produced by Th17 cells and T follicular helper (Tfh) cells. In a cross-sectional study including PV patients and healthy controls, peripheral blood mononuclear cells (PBMC) were analysed for CD4+ T cell subsets using flow cytometry as well as for cytokine levels by ELISA. Patients with the neuromuscular disease myasthenia gravis (MG) were included as a further unrelated antibody-mediated autoimmune disease. So far, our results suggest higher frequencies of IL-21-producing T cells in PBMC of PV patients after *in vitro* stimulation. Of note, for the first time we could detect Dsg3-specific autoreactive T cells producing IL-21 upon ex vivo stimulation with Dsg3 by ELISpotassay. In accordance with the increased plasma levels of IL-21 in PV patients the frequencies of circulating Tfh cells (defined as CD4+CXCR5+ T cells) as well as Th17 cells were significantly elevated in PV. Ongoing experiments aim to further specify the functional capacity of Tfh cells and Th17 cells in PV: i) CD4+CXCR5+ T cells are cocultured with CD19+ B cells in order to test whether Tfh cells in PV can induce Dsg3-specific auto-ab production in an IL-21-dependent manner, ii) Tfh cells in PV are further subdivided into Tfh-Th1, Tfh-Th2 and Tfh-Th17 groups according to the differential expression of CXCR3 and CCR6 on CD4+CXCR5+ T cells. The more defined characterization of IL-21-producing cells will lead to a better understanding of the pathogenesis of PV and finally it may contribute to novel, pathogenesis-driven therapeutic options in PV in the future.

P137 (O01/03)

9-cis-retinoic acid modulates dendritic cell differentiation to generate a Treg inducing phenotype – an important function of Osteopontin

L. F. Kraus, N. Scheurmann, D. Frenzel and J. M. Weiss *Ulm University, Dermatology and Allergy, 89081 Ulm, Germany*

9-cis-retinoic acid (9cisRA, Alitretinoin) is a high-affinity pan-agonist for the retinoic acid receptors (RAR) and retinoid X receptors (RXR). 9cisRA is effective for treating chronic hand eczema, which is often associated with delayed type allergy. There is limited data how 9cisRA exerts anti-inflammatory functions in the skin immune system. We previously described that Osteopontin (OPN), which has modulating cytokine functions in autoimmunity and allergy, is strongly expressed by immune cells in the inflammatory infiltrate of contact dermatitis. We here investigated the functional effects of 9cisRA on dendritic cell (DC) T cell interactions in the context of allergic contact hypersensitivity (CHS) and a possible modulatory role of OPN.

Murine bone marrow derived DC were cultured by standard protocol in the presence of different concentrations of 9cisRA. We found that in comparison to untreated DC the highly CD11c expressing DC that were differentiated from murine bone marrow in the presence of 9cisRA (9cisDC) expressed less MHC-II, CD44 and CD86. In contrast the co-inhibitory PD1-L was induced on 9cisDC. Further, 9cisDC had an altered pattern of cytokine and chemokine expression, secreting less IL-1beta, IL-12p70, CXCL9, CXCL10 and CCL-1, but highly secreted OPN. To investigate the functional characteristics of 9cisDC we performed allogeneic mixed lymphocyte reactions. 9cisDC were less potent in stimulating T cell proliferation, however, they potently converted naïve T cells into CD4+/Foxp3+/CD25+ Treg cells. Such co-cultures contained less IL-2, IL-4, IL-5 IL-10, IL-13, IL-17A and IFN-gamma compared to controls. Speculating that the strongly induced OPN from 9cisDC could be of role for Treg conversion we performed criss-cross allogeneic MLR with OPN deficient DC and T cells. Interestingly, we found that 9cisDC from OPN deficient mice are compromised in their Treg inducing function. Upon addition of recombinant OPN, OPN$^{-/-}$ 9cisDC regained their Treg converting potential. Finally, *in vivo*, we tested whether 9cisDC were able to modulate established antigen specific CHS. When TNCB sensitized mice were treated with TNBS loaded 9cisDC 6 days after sensitization they inhibited CHS response compared to mice injected with untreated TNBS loaded DC. Further, 9cisDC treated TNCB sensitized mice showed elevated numbers of Tregs in skin draining lymph nodes 48 h after antigen challenge. Again, *in vivo*, OPN$^{-/-}$ 9cisDC were less potent Treg inducers.

In conclusion our findings propose that 9cisRA modulates DC toward a phenotype that is able to suppress established contact allergy through the induction of Tregs, a mechanism that is at least partially modulated by OPN.

P138

Cutaneous RANK/RANKL and S100A8/A9 signaling controls innate and adaptive anti-viral immunity during Herpes simplex virus infection

L. Klenner[1], W. Hafezi[2], T. Vogl[3], J. Roth[3], T. A. Luger[1], J. Kühn[2] and K. Loser[1] [1]*University of Muenster, Department of Dermatology, 48149 Muenster, Germany;* [2]*University of Muenster, Department of Medical Microbiology, 48149 Muenster, Germany;* [3]*University of Muenster, Institute of Immunology, 48149 Muenster, Germany*

Skin infections are controlled by the immune system and since the receptor RANK as well as its ligand RANKL are up-regulated in virus infected skin, we analyzed whether this signaling pathway might be involved in the regulation of cutaneous anti-viral immunity. In a mouse model of epicutaneous Herpes simplex virus (HSV) infection of K14-RANKL transgenic (tg) mice overexpressing RANKL in keratinocytes, we have previously shown that RANKL signaling expanded virus-specific CD8+ cytotoxic T lymphocytes (CTL) by preventing virus-induced apoptosis of epidermal Langerhans cells and thereby, improving the transport of viral antigens to regional lymph nodes. Notably, two intralesional injections of recombinant RANKL protein were sufficient to induce the protective effect in HSV-infected wildtype (wt) mice. Since the damage-associated molecular pattern (DAMP) molecules S100A8 and S100A9 are essential for the activation of CD8+ T cells as we have demonstrated in systemic autoimmunity, we next investigated the impact of S100A8 and S100A9 signaling on anti-viral immune responses. Therefore, K14-RANKL tg mice were bred to S100A9 deficient animals lacking both, S100A8 and S100A9 on protein level. Subsequently, double-mutants were infected with HSV. Notably, the absence of S100A8/A9 proteins abrogated the protective effect of cutaneous RANKL signaling since K14-RANKL tg × S100A9$^{-/-}$ mice and S100A9$^{-/-}$ controls showed a similar disease progression. Moreover, the numbers of CTL as well as the expression of cytolytic markers in CD8+ T cells from lesional skin and regional lymph nodes of HSV-infected K14-RANKL tg x S100A9$^{-/-}$ mice were comparable to S100A9$^{-/-}$ controls suggesting that S100A8 and S100A9 are required as innate amplifiers for the RANK/RANKL-mediated induction of MHC class I-mediated anti-viral immunity. Interestingly, when analyzing early time points after infection we could show that besides increasing virus-specific CTL, RANK/RANKL signaling up-regulated the levels of innate effector cells, like innate lymphoid cells (ILC), in HSV-infected K14- RANKL tg mice. ILC have been implicated in protecting the host from infection, mediating tissue remodeling or repair, and improving the integrity as well as function of epithelial barriers. At day 2–4 after HSV-infection the numbers of CD4-CD8- CD19-CD11c-Sca1+Thy1+T-bet+ ILC1 as well as CD4-CD8-CD19-CD11c-Ror-γt +CD127+IL-1R+IL-23R+ ILC3 were 2–3 fold increased in lesional skin from K14- RANKL tg mice as compared to wt controls. Of note, these increased numbers of tissue-protective ILC were not detectable in HSV-infected skin from K14-RANKL tg x S100A9$^{-/-}$ double mutants, suggesting that in addition to adaptive anti-viral immunity RANK/RANKL and S100A8/A9 signaling might also act synergistically in the induction of protective innate responses during HSV infection. Thus, our data indicate that in cutaneous viral infections an interplay of RANK/RANKL and S100A8/A9 signaling might be critical for the induction of innate and adaptive antiviral immune responses.

P139

Redox-mediated modulation of immune activation through toll-like receptors (TLR)

B. Bauer, H. Braumüller, T. Wieder and M. Röcken *University Medical Center Tuebingen, Department of Dermatology, 72076 Tuebingen, Germany*

Inflammatory autoimmune diseases such as psoriasis or multiple sclerosis (MS) have been shown to be associated with aberrant induction of interleukin (IL-) 12 and IL-23- producing dendritic cells (DC),

resulting in pathogenic Th1 and Th17 cell responses. Dimethylfumarate (DMF) is a small molecule that directly improves MS as well as psoriasis by generating IL-10 producing type II DCs that promote Th2 cells instead of proinflammatory Th1/Th17 cells. This immune deviation may be caused by oxidative stress, as DMF treatment results in a depletion of the reduced form of intracellular glutathione (GSH), the cells' most important scavenger of reactive oxygen species (ROS). As previous data suggest that ROS play an important role in the regulation of immune responses, the aim of this study is to investigate the impact of the redox system on activation of distinct immune pathways. Here, hemeoxygenase-1 (HO-1) and the STAT1 signaling pathway are important players as they are known to modify the expression of IL-23 and IL-12 upon GSH depletion. In order to investigate this in more detail, we studied the interaction between glutathione depletion by DMF on IL-12, IL-23 and HO-1 expression as well as on the phosphorylation of STAT1 after TLR4 stimulation with lipopolysaccharides. First results showed that DMF treatment decreased intracellular GSH and simultaneously IL-23 levels. Both effects were reversed in the presence of the antioxidant *N*-acetylcysteine (NAC). To specifically analyze the redox-mediated modulation of immune activation without other possible interfering effects of DMF, we additionally isolated bone marrow derived dendritic cells (BMDC) from cystine/glutamate antiporter knockout mice. This genetic knockout of the cystine/glutamate antiporter results in reduced levels of glutathione and leads to persistent ROS stress. Moreover, analysis of BMDC from these mice will unravel whether genetic knockout of the glutathione pathway mimics the effect of DMF on cytokine production and HO-1 as well as on the STAT1 signaling pathway. These experiments will demonstrate whether GSH depletion and chronic ROS stress are sufficient to transform dendritic cells to a DC type II phenotype or whether other targets of DMF play a role in immune deviation to the anti-inflammatory Th2 cell response.

P140

Hydroxyethyl starch nanocapsules as a potent drug-delivery system for targeting of CD4+CD25+ T cells with different IL-2 receptor affinities

M. P. Domogalla[1,2], S. U. Frick[1], G. Baier[2], F. Wurm[2], V. Mailänder[1,2], K. Landfester[2] and K. Steinbrink[1] *[1]University Medical Center Mainz, Department of Dermatology, 55131 Mainz; [2]Max-Planck-Institute for Polymer Research, 55131 Mainz*

Due to an increase of efficiency and a reduction of side effects, cell-type specific drug delivery by use of nanoparticles is a promising approach for induction of efficient anti-tumor responses in cancer immunotherapy. In contrast to targeting antigen presenting cells (APC) like macrophages or dendritic cells which has become a common approach, addressing T cells remains an obstacle owing to low endocytic activity. In the present study, we used IL-2 functionalized hydroxyethyl starch (HES) nanocapsules (NC) generated by miniemulsion for targeting of CD4+CD25+ T cells. For IL-2 functionalization, HES-NC were conjugated with dibenzocyclooctyne (DBCO) and azide functionalized IL-2 was covalently linked to the NC surface by copper-free click reaction (HES-D-IL-2). In flow cytometry studies with human CD4+CD25high T cells, HES-D-IL-2 NC demonstrated an enhanced uptake and IL-2 induced proliferation compared to DBCO functionalized control capsules (HES-D). The uptake was confirmed by laser scanning microscopy that revealed internalization of the HES-D-IL-2 NC whereas the HES-D capsules were rather attached to the cell membrane. In order to verify the specificity of the observed uptake, CD4+CD25high T cells were additionally incubated with the chimeric monoclonal anti-CD25 antibody Simulec (basiliximab) to block the alpha chain of the IL-2 receptor which prevents binding and internalization of IL-2. In contrast to HES-D binding that was not altered, uptake and proliferation of human CD4+CD25high induced by HES-D-IL-2 was inhibited by IL-2 receptor blockade. Furthermore, flow cytometry analysis revealed that HES-D-IL-2 were preferably taken up by CD25high and CD25+ T cells in comparison to CD25- T cells. Moreover, in order to target T cells with different IL-2 receptor affinities, we introduced different, quantifiable amounts of IL-2 on the NC surface and generated NC with twofold (HES-D-IL-2/2) and tenfold (HES-D-IL-2/10) reduced IL-2 quantities. Intriguingly, the uptake of IL-2 functionalized NC and induced proliferation of human CD4+CD25high T cells was significantly reduced when less IL-2 was present on the capsules surface. To investigate the impact of HES-D-IL-2 *in vivo*, control HES-D and HES-D-IL-2 NC were intravenously injected into wild type C57BL/6 mice. After 24 h and enhanced uptake of HES-D-IL-2 NC was observed in lymph node-related CD4+CD25+ T cells while no relevant uptake in B cells (B220+) dendritic cells (CD11c+) and myeloid cells (CD11b+, F4/80+) was detected.

In summary, we generated IL-2 functionalized nanocapsules with controlled and quantified amounts of IL-2 on the surface that allowed for efficient murine and human CD4+CD25+ T cell targeting with various IL-2 receptor affinities *in vitro* and *in vivo*. The technique may be translated to other cytokine-related targeting approaches and may be a promising concept for T cell-based immunotherapies.

P141

Cell type specific expression and induction of IL-33 mRNA and protein

J. Brück, A. Schlögl, J. Holstein, K. Meier, B. Fehrenbacher, K. Ghoreschi and A. S. Yazdi *University of Tuebingen, Department of Dermatology, 72076 Tuebingen, Germany*

Jürgen Brück, Alexandra Schlögl, Julia Holstein, Katharina Meier, Birgit Fehrenbacher, Kamran Ghoreschi, Amir S. Yazdi

Key mediators of innate immunity during skin inflammation are cytokines of the interleukin-1 (IL-1) family. While IL-1α and IL-18 are well characterized and are activated via inflammasome activation, the activation of IL-33 is still cryptic and in contrast, IL-33 is claimed to be inactivated by caspases. Recent data suggest that IL-33 acts as an alarm or danger signal and is released after cell damage. Besides binding to its specific receptor ST2, IL-33 can be present in the nucleus and in the cytosol.

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

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

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

P142

Impact of fumaric acid esters on T cell subsets in patients with psoriasis

S. Riemann[1], B. Greene[2], M. Hertl[1] and C. Möbs[1] *[1]Philipps University Marburg, Department of Dermatology and Allergology, 35043 Marburg, Germany; [2]Philipps University Marburg, Institute of Medical Biometry and Epidemiology, 35037 Marburg, Germany*

Background: Psoriasis is a frequent, T cell-mediated, chronic inflammatory skin disease. In cases of resistance to both topical treatment and phototherapy, treatment with fumaric acid esters (FAE) has shown to be effective. Several immunomodulatory mechanisms leading to clinical improvement of psoriasis have been discussed, such as heme oxygenase-1 (HO-1) induction, anti-angionetic effects, T helper 1 (Th) 1 to Th2 cell shift or general T cell suppression. Nevertheless, the precise mode of action and the main active compound of FAE remain unclear.

Methods: Psoriasis patients (n = 13) treated with FAE were followed from initiation of therapy over a period of up to 12 weeks. ELISPOT analysis was used to quantify the frequencies of IFN-γ-, IL-17-, IL-5-, IL-10-secreting cells in peripheral blood, representing the frequencies of Th1, Th17, Th2 and regulatory T (Treg) cells in patients with psoriasis. Furthermore, the *in vitro* influence of distinct FAE compounds (fumaric acid, FA; monomethyl fumarate, MMF; and dimethyl fumarate, DMF) on peripheral blood mononuclear cells (PBMC) was investigated after stimulation with anti-CD3, anti-CD3/CD28, phytohemagglutinin (PHA) and the antimicrobial peptide LL37.

Results: Frequencies of IFN-γ-, IL-17-, IL-5-, IL-10- and IFN-γ/IL-17 double-positive T cells after unspecific stimulation did not differ significantly between psoriasis patients before initiation of therapy and healthy controls. In contrast, after simulation of PBMC with LL37, which has been proposed as an autoantigen in psoriasis, LL37- specific IFN-γ-positive T cells could be detected in one third of the psoriasis cohort but not in healthy individuals. Longitudinal analysis of FAE-treated psoriasis patients revealed decreasing numbers of T cells, especially in the IFN-γ-positive T cell subset. Furthermore, the impact of different FAE compounds on PBMC was examined. *In vitro* treatment of PBMC with DMF showed a significant and concentration-dependent reduction of T cells in psoriasis patients and the healthy control cohort. In contrast, incubation of cells with FA or MMF led only to a significant decrease in the IL-10- positive T cell subsets.

Conclusion: Our results suggest that treatment of psoriasis patients with FAE leads to early immunomodulatory effects on the T cellular level. Interestingly, FAE did not induce IL-10-positive Treg cells in the peripheral blood of patients as shown for treatment with TNF-α blockers. FAE rather seem to directly affect T cells, since our *in vitro* results revealed that DMF strongly reduced T cell numbers after activation. Furthermore, we could detect LL37-specific T cells in one third of our patient cohort but not in healthy individuals emphasizing its role as an autoantigen at least in a subset of patients with psoriasis.

P143

PI3K? inhibition effectively ameliorates experimental epidermolysis bullosa acquisita

H. Koga[1,3], N. Godessart[2], A. Kasprick[1], K. Matsumoto[1], E. Schmidt[1], R. J. Ludwig[1] and C. Balagué[2] *[1]Institute of Experimental Dermatology, University of Luebeck, 23562 Luebeck, Germany; [2]Almirall R & D, Sant Feliu de Llobregat, 08980 Barcelona, Spain; [3]Kurume University School of Medicine, Department of Dermatology, 8300011 Kurume, Japan*

Epidermolysis bullosa acquisita (EBA) is a rare severe bullous disease of high unmet medical need. Patients develop loss of tolerance to type VII collagen (COL7), a skin basement membrane component, resulting in the production of autoantibodies. Through binding to COL7 and subsequent activation of myeloid cells (i.e. neutrophils), anti-COL7 antibodies cause chronic muco-cutaneous blistering in EBA. Therapies aimed at controlling the humoral response and/or neutrophil activation may therefore provide a new therapeutic option. We have investigated the therapeutic potential of a novel isoform-selective PI3Kδ inhibitor, named LAS191954, in the treatment of EBA. PI3Kδ is expressed in different immune cells and has been shown to be essential for B cell development and function.

We first assessed the effect of PI3Kδ inhibition on primary human PMNs activation *in vitro* by measuring the production of reactive oxygen species (ROS) induced by binding to COL7-antiCOL7 immune complexes (IC). ROS release by human IC-activated PMNs *in vitro* was potently and dose-dependently inhibited by the compound with nanomolar IC50s. By contrast, methylprednisolone inhibited ICinduced ROS release only at high micromolar concentrations.

We next assessed the effect of PI3Kδ inhibition in an *in vitro* blistering model. Human skin cryosections were incubated with COL7 immune complexes-bound PMNs in the presence or absence of the compound. LAS191954 dose-dependently prevented the dermal -epidermal separation induced by IC-mediated activation of PMN.

The effect of pharmacological inhibition of PI3Kδ on the clinical manifestations of EBA was assessed in an experimental EBA mouse model using a curative scheme. In this model, disease was induced by immunization with COL7 and manifested as areas of erythema and/or crusts. After the onset of clinical EBA manifestations, mice were allocated to different treatment groups (vehicle or LAS191954 at 1 and 3 mg/kg doses) and administered daily for 6 weeks by oral gavage. In addition, a group treated with a high dose of methylprednisolone (MP) (20 mg/kg) was used as a comparator. Changes in clinical EBA manifestations were monitored weekly. In vehicle-treated mice, disease severity increased 2-fold during the observation period of 6 weeks. Compared to vehicle-treated mice, MP treatment halted the progression of the disease at the end of the observation period. By contrast, LAS191954 at the highest dose tested reduced the initial disease severity by half, effectively ameliorating inflammation and blistering.

Collectively, our data suggest that PI3Kδ is involved in neutrophil functions that underlie skin damage in EBA, and that specific PI3Kδ inhibition may be of therapeutic benefit in the treatment of EBA and related autoimmune bullous diseases.

P144

Gene-environment interaction controls ANA production in mice

A. Vorobyev[1], Y. Gupta[1], H. Koga[2], P. Kouki[1], H. Körber-Ahrens[1], S. Ibrahim[1] and R. J. Ludwig[1] *[1]Luebeck Institute of Experimental Dermatology, University Clinic of Schleswig- Holstein, 23538 Luebeck, Germany; [2]Kurume University School of Medicine, Department of Dermatology, 830-0011 Kurume, Japan*

Autoimmune diseases have become a major clinical burden, and despite significantly improved treatment options, unspecific immunosuppression is still the backbone of their treatment. This, however, leads to severe side effects and partially contributes to the increased mortality of patients. Therefore, targeted, pathogenesis-oriented treatments could improve both, efficacy and safety of treatment of patients with autoimmune diseases. During the past years genome-wide association studies (GWAS) have added to our understanding of the pathogenesis in autoimmune diseases. Yet, identified susceptibility genes by far do not fully explain disease susceptibility. The so-called 'missing heritability' may be explained by environmental factors, i.e. diet, which may modify susceptibility to autoimmune diseases. To address this missing heritability experimentally, over 1200 mice of an autoimmuneprone intercross mouse line were fed 3 different diets (calorie reduced, control and western diet; n > 350 mice/group). Of note, while we identified quantitative trait loci (QTL) associated with the presence of anti-nuclear antibodies (ANA), diet had by far a greater impact on ANA formation. In detail, 42% of mice on control diet developed ANA during the 5 month observation period. In calorie-restricted mice, ANA were observed in 30% of the animals, while 67% of mice on western diet showed ANA. Also, the endpoint titers were higher in western compared to other diets. When using diet as an interactive covariate in the QTL-mapping, several of these QTLs were 'diet-sensitive'; i.e. when considering diet as a covariate, some new QTLs were observed, while others became irrelevant, exemplified by QTLs on chromosomes 2, 11, 13. Our findings deliver evidence for gene-environment interaction control of ANA production in mice. At this stage we could narrow down the identified 'dietsensitive' QTLs to 0.5–4 Mb. By further analysis we aim to identify the single genes controlling different phenotypes and prove their relevance *in vivo*. This will provide detailed insights into pathogenesis of autoimmunity, as well as gene-environment interaction.

P145

Platelets have immunoregulatory function on the differentiation process of CD4+ T cells

N. Zimmer, S. A. Hahn and A. Tuettenberg *University Medical Center, Johannes Gutenberg University, Dermatology, 55131 Mainz, Germany*

The main function of platelets is to initiate homeostasis. In case of inflammation platelets are rapidly deployed to sites of infection and modulate inflammatory processes by interacting with leukocytes. However, detailed information on their interaction is limited. Glycoprotein A repetitions predominant (GARP), an activation marker on the surface of human regulatory T cells (Treg), was first described on platelets. On both cell types GARP serves as a transporter for TGF-β. By modulating bioavailability of TGF-β, GARP is involved in the regulation of peripheral immune responses. We have recently shown that soluble GARP (sGARP) detached from the membrane of Treg has strong anti-inflammatory and regulatory properties *in vitro* as well as *in vivo* and leads to induction of peripheral Treg.

In the present study, we analyzed the effect of platelets on the differentiation of CD4+ T cells according to GARP. Furthermore, we investigated the supernatant of activated platelets for the presence of sGARP. Briefly, we cultured CD4+ T cells together with different ratios of platelets and platelets' supernatant and analyzed the alteration in phenotype. We found that platelets inhibit the proliferation of CD4+ T cells in a dose dependent manner and lead to an induction of Foxp3. Furthermore preliminary results show that there is a reduced cytokine production in CD4+ T cells cultured in presence of platelets. These phenotypical changes give a first hint that there could be an induction of Treg in the presence of platelets. Further studies will investigate the impact of GARP in platelet-mediated regulation of CD4+ T cells.

In conclusion, our data give first evidence that platelets are involved in the induction of peripheral Treg. This aspect is of importance in diseases like cancer where increase in circulating platelets (thrombocytosis) is recognized as an independent risk factor of bad prognosis and metastasis.

P146

An Fc-optimized CD19 antibody as treatment option for lymphoid B-cell malignancies

U. J. Seidel[1], L. Grosse-Hovest[2,3], P. Schlegel[1], M. Hofmann[2,3], F. Vogt[3,5], F. R. Schuster[4], R. Meisel[4], K. Witte[1], S. Aulwurm[2,3], E. Pyz[3], H. Rammensee[3,5], G. Jung[3,5], R. Handgretinger[1,5] and P. Lang[1,5] *[1]University Children's Hospital Tuebingen, Department of General Paediatrics, Oncology/Haematology, 72076 Tuebingen, Germany; [2]SYNIMMUNE GmbH, 72076 Tuebingen, Germany; [3]University of Tuebingen, Interfaculty Institute for Cell Biology, Department of Immunology, 72076 Tuebingen, Germany; [4]Heinrich Heine University, Department of Paediatric Oncology, Haematology and Immunology, Düsseldorf, Germany; [5]German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), 72076 Tuebingen, Germany*

B-cell malignancies include different types of lymphomas and leukaemias. The different entities occur upon disrupted regulation of B-cell differentiation and activation and may result into aggressive types of B-cell lymphomas and leukaemias requiring systemic therapy. Current treatment options include radiation, chemotherapy and immunotherapy with antibodies like rituximab targeting CD20. Another potential immunotherapeutic target of B-cell malignancies is CD19, which is highly expressed in cutaneous B cell lymphomas (CBCL) or B-precursor acute lymphoblastic leukemia (BCP-ALL).

We developed a chimeric Fc-optimized third-generation CD19 antibody (4G7SDIE) and produced it in pharmaceutical quality. This antibody mediates markedly enhanced antibody dependent cellular cytotoxicity (ADCC) through its improved capability to recruit FcγRIIIa bearing effector cells. In *in vitro* cytotoxicity assays NK cells and $\gamma\delta$ T cells were identified as main effector cell populations. PBMC of healthy volunteers and pediatric B-lineage ALL patients mediated enhanced lysis of leukemic blasts *in vitro*. A positive correlation between CD19 surface expression levels and 4G7SDIE mediated lysis was observed. The FcγRIIIa-V158F-polymorphism had no influence on ADCC mediated by 4G7SDIE whereas this phenomenon has been described for rituximab. In 9/14 pediatric B-lineage ALL patients treated with 4G7SDIE a reduction or elimination of minimal residual disease was observed. Side effects were very mild including headache and fever. Furthermore, a complete CD19+ and CD20+ B cell depletion was observed in all patients during treatment.

In conclusion, promising anti-leukemic effects of the 4G7SDIE antibody have been observed *in vitro* and *in vivo*. 4G7SDIE might be beneficial not only for BCP-ALL but also for patients with aggressive CBCL in which rituximab-based therapies failed to induce enduring remission.

P147

Scurfy mice spontaneously develop autoantibodies with reactivity to skin antigens

S. Häberle, X. Wei, A. H. Enk and E. N. Hadaschik *University Hospital Heidelberg, Department of Dermatology, 69120 Heidelberg, Germany*

In Scurfy mice a missense mutation in the foxp3 gene causes loss of function of regulatory T cells. The expansion of autoreactive CD4+ T cells leads to multiorgan autoimmune inflammation. In addition scurfy mice have elevated levels of IgE and IgG autoantibodies in their sera. We aimed to analyze the specificity of these autoantibodies to autoantigens in the skin.

We used indirect immunofluorescence staining to characterize the different autoantibodies from sera of scurfy mice and WT littermate controls. Frozen palate slices of wildtype (WT) mice were used for indirect immunofluorescence staining with sera from sick scurfy mice or WT littermate controls as the primary antibody followed by detection by an anti-mouse IgG secondary antibody. Sera of scurfy mice showed positive staining in the epidermis with different staining patterns, whereas sera of WT mice did not show a specific staining. The autoantibodies from scurfy sera showed either a linear staining indicative of recognition of proteins of the basal membrane or a reticular staining indicative for desmosomal autoantigens. To identify tissue-bound autoantibodies we used direct immunofluorescence on frozen palate slices of sick scurfy mice. We found deposits of autoantibodies in the skin of scurfy but not WT mice. An enzyme linked immunosorbent assay (ELISA) revealed that the majority of sera of scurfy mice contained autoantibodies specific for desmoglein 3, the known antigen for the blistering autoimmune disease pemphigus vulgaris.

In summary we show that scurfy mice spontaneously develop autoantibodies with reactivity to skin and that some scurfy mice have autoantibodies specific for desmoglein 3.

P148

The role of the PAR4/Cathepsin G (CatG) pathway in the regulation of neutrophil-platelet aggregations (NPAs)

K. Plesser[1], M. Feld[1], F. Smits[1], J. Buddenkotte[1], M. Fastrich[1], M. Spelleken[2], C. Hillgruber[3], M. Elvers[2] and B. Homey[1] *[1]Heinrich-Heine-University of Duesseldorf, Department of Dermatology, 40225 Duesseldorf, Germany; [2]Heinrich-Heine-University of Duesseldorf, Molecular Hemostasis Institute for Hemostasis, Hemotherapy & Transfusion Medicine, 40225 Duesseldorf, Germany; [3]University of Muenster, Department of Dermatology, 48149 Muenster, Germany*

Introduction: NPAs are important for the regulation of inflammatory responses in several tissues. During inflammation platelets are essential for the recruitment of neutrophils to the site of inflammation. Protease-activated receptors (PARs) belong to the family of G-protein coupled receptors and are expressed in all tissues. Pathophysiologic mechanisms including vascular injury, acute inflammation, or allergen exposure cause the release of several proteases that mediate the activation of PARs. Here, we aim to understand the role of the PAR4/Cathepsin G (CatG) pathway in platelets. Further, we attempt to unravel its effect on NPAs and to determine its involvement in the progression of vascular inflammation, such as leukocytoclastic vasculitis (LcV).

Materials and Methods: *In vitro*, we established an aggregation assay with primary neutrophils and platelets isolated from healthy human whole blood. Platelets were stimulated with CatG and analysed for their ability to form aggregates with neutrophils after a short period of co-incubation. Ex vivo, we evaluated the role of NPA in vascular inflammation. To achieve this, we measured the abundance of NPAs in fresh whole blood of LcV patients and healthy donors. In an *in vivo* model of vasculitis, the reverse passive Arthus reaction (RPA), we aim to validate our primary findings. Briefly, the RPA is induced in C57Bl/6 wild type and Par4$^{-/-}$ mice by (1) intravenous injection of BSA/Evans Blue and by (2) intradermal injection of anti-BSA into the back skin. We determined neutrophil tissue infiltration (Myeloperoxidase (MPO) tissue level) and edema formation (weight of biopsy) as a measure of inflammation in RPA mice. In addition to this we did a full blood count to determine leukocyte distribution and to identify the abundance of NPAs in the whole blood of wild type and Par4$^{-/-}$ mice after 4 h of RPA.

Results: Stimulation of platelets with CatG revealed a significant increase in NPA. This effect is in part mediated by PAR4, as blocking PAR4 with an antagonist severely decreases NPA induction by CatG. Patients with LcV show strongly increased formation of NPAs compared to healthy donors. With our *in vivo* model of vasculitis we confirmed our *in vitro* findings and found significant increased NPA counts in whole blood of wild type mice, but not in Par4$^{-/-}$, after 4 h of RPA compared to untreated mice. Also compared to the wild-type situation, Par4$^{-/-}$ mice show an increase in inflammation when RPA has been induced. Differential blood count analysis revealed a higher percentage of granulocytes and monocytes and a lower relative abundance of lymphocytes in the whole blood of RPA mice compared to untreated mice.

Conclusions: *In vitro*, we assessed the effect of CatG on neutrophil-platelet interactions. In addition, we also determined the important role of PAR4 on the formation of NPAs *in vitro* and *in vivo*. Further, we identified the formation of NPAs as an important marker for vasculitis ex vivo in humans as well as in our *in vivo* model. Inflammation in Par4$^{-/-}$ mice after 4 h of RPA is severely increased compared to wild type C57Bl/6, indicating an anti-inflammatory effect of the PAR4/CatG pathway in mice. Differential blood count analysis revealed a redistribution of leukocyte subtypes in the whole blood of mice after inflammation compared to healthy murine blood.

P149

The Aryl Hydrocarbon Receptor mediates sensing of Staphylococcus epidermidis in human keratinocytes

F. Rademacher, B. Hesse, M. Simanski, R. Gläser and J. Harder *University of Kiel, Department of Dermatology, 24105 Kiel*

Staphylococcus epidermidis (S. epidermidis) is an abundant member of the human cutaneous microbiota. There is increasing evidence that sensing of S. epidermidis by keratinocytes is important to strengthen cutaneous innate defense through the induction of defense mediators such as IL-1. The underlying signal transduction pathways mediating S. epidermidis recognition by human keratinocytes have not yet been fully elucidated.

Here we show for the first time that stimulation of human primary keratinocytes with S. epidermidis led to activation of the aryl hydrocarbon receptor (AhR). This was demonstrated by activation of an AhR luciferase gene reporter as well as by induction of the AhR-regulated CYP1A1 gene in keratinocytes stimulated with living S. epidermidis. S. epidermidis-mediated CYP1A1 gene induction was blocked by coincubation of the keratinocytes with the specific AhR inhibitor CH-223191 confirming the activation of the AhR by S. epidermidis.

CH-223191 also significantly decreased the S. epidermidis-induced IL-1beta gene and protein expression in keratinocytes. To further assess the role of the AhR for the S. epidermidis-induced IL-1beta expression we down-regulated AhR expression in human primary keratinocytes using AhR-specific siRNA. Knockdown of AhR expression by siRNA confirmed the participation of the AHR in the induction of IL-1beta in human keratinocytes stimulated with S. epidermidis. Finally we used a 3D skin equivalent to evaluate the role of the AhR in sensing S. epidermidis. Infection of the 3D skin equivalent with S. epidermidis induced CYP1A1 expression, a response that was inhibited by co-treatment with the specific AhR inhibitor CH-223191. Similarly, IL-1beta gene expression and protein expression was induced by S. epidermidis in the 3D skin equivalent and this induction was decreased by the AhR inhibitor CH-223191.

In conclusion, our data indicate (1) that the AhR mediates the recognition of S. epidermidis in human keratinocytes and (2) that AhR activation by S. epidermidis is required for full induction of S. epidermidis-induced IL-1beta expression in human keratinocytes. These data imply a novel role of the AhR as putative pattern recognition receptor of S. epidermidis in human keratinocytes.

P150 (O02/06)

Highly abundant T cell receptors are involved in the skin blistering disease Epidermolysis Bullosa Acquisita

M. Niebuhr[1], A. Fähnrich[1], S. Maass[1], R. J. Ludwig[2], J. Westermann[1] and K. Kalies[1] *[1]University of Luebeck, Institute of Anatomy, 23562 Luebeck, Germany; [2]University of Luebeck, Department of Dermatology, 23562 Luebeck, Germany*

Epidermolysis bullosa acquisita (EBA) is an organ-specific autoimmune disease and associated with skin-lesions and subepidermal blisters. The disease progression is characterized by the production of complement activating autoantibodies targeting type VII collagen, an integral element of the dermal-epidermal junction. For a better pathophysiological understanding of EBA, an experimental model has been established by immunizing mice with murine type VII collagen (mCol7). In this mouse model, the induction and binding of anti-mCol7 specific auto-antibodies induce a proinflammatory environment, leading to separation of the dermal-epidermal junction. Thereby specific and well-defined skin areas, e.g. ears, eyes and snout, are prone to blister development. During onset and progression of experimental EBA, T cells were identified as important mediators in the mCol7-specific immune response.

In the present study, we focus on the identification of precise factors promoting blister formation at affected skin sites. Immunohistochemical and gene expression analysis showed an accumulation of T cells in skin lesions and an elevated expression of the Th1 cytokine IFNγ. To address the question, if accumulated T cells are autoreactive and directed against mCol7, we identified the T cell receptor (TCR) sequences by next generation sequencing. Comparing the TCR sequences in germinal centers of draining lymph nodes to them in skin lesions, we found over 60% of the highly abundant TCR sequences to be shared between both compartments. Moreover, we observed a high TCR sequence homology between individual mice. This data indicates that T cells extravasating in affected skin lesions are predominantly autoreactive and directed against the autoantigen mCol7. We hypothesize that the presence of these mCol7-specific T cells is of critical importance for triggering skin lesions. In addition, we are further investigating the TCR composition within the mCol7-specific T cells, focussing on possibly preferred TCR segment-usage, crucial for the break of tolerance to the autoantigen mCol7. Such a finding might provide deeper insights in the disease-supporting role of T cells in immunization induced EBA and potentially enables an approach for disease attenuation in mice.

P151

Omalizumab: an anti-IgE antibody binding to CD16 (FcγRIIIa)

S. Oehrl, M. Maas, T. Döbel, S. Meisel, S. Pezer and K. Schäkel *University Hospital Heidelberg, Department of Dermatology, 69120 Heidelberg, Germany*

Omalizumab (Xolair®) is a humanized IgG1κ monoclonal antibody that selectively binds immunoglobulin E (IgE) and was initially licensed for the treatment of allergic asthma. Recently, omalizumab is also approved for the treatment of chronic idiopathic urticaria which can occur independent of IgE. This suggests a mechanism for omalizumab besides IgE depletion; however other mechanisms are still poorly understood. IgG can exert effects by interaction with different Fcγ receptors (FcγR) via their Fc portion. Engagement of these receptors can induce inhibitory signals via different mechansims. It has been described that the low affinity IgG receptor CD16a (FcγRIIIa) can induce inhibitors signal via its immunoreceptor tyrosinebased activation motif (ITAM). CD16a strongly binds immune complexes and we therefore hypothesized that omalizumab-IgE immune complexes can bind to CD16a on immune cells, thereby leading to inhibition of these cells. First we analyzed by size-exclusion chromatography if co-incubation of omalizumab and IgE results in the formation of immune complexes. These immune complexes could be purified for our experiments. When we analyzed the binding of preformed omalizumab-IgE complexes to CD16 expressed by a subtype of blood dendritic cells (slanDC), we found that not only the immune complexes but also monomeric omalizumab alone could bind to CD16. To confirm these findings and to analyze if omalizumab can also bind to other FcγR, we transfected Jurkat cells with either CD16a, CD32a (FcγRIIa) or CD32b (FcγRIIb). However, in our system we could solely detect a binding to CD16atransfected Jurkat cells but not to Jurkats transfected with other FcγR. It now needs further investigation if the binding of omalizumab to CD16-expressing cells can result in inhibitory intracellular signaling which potentially could explain IgE-independent effects on the immune system.

P152 (O04/04)

Do tissue-resident macrophages in human skin derive from intradermal CD34+ progenitor cells?

J. Gherardini[1], Y. Uchida[1,2], J. Chéret[1,3], M. Alam[1], M. Bertolini[1,3] and R. Paus[1,4] *[1]University of Muenster, Dermatology, Muenster, Germany; [2]Kagoshima University Graduate School of Medical and Dental Sciences, Dermatology, Kagoshima, Japan; [3]Monasterium Laboratory, Skin & Hair Research Solutions UG, Muenster, Gemany; [4]University of Manchester, Centre for Dermatology Research, Institute of Inflammation and Repair, Manchester, United Kingdom*

In murine skin, self-renewing tissue-resident macrophages (trMAC), rather than MACs that have differentiated from extravasated monocytes, are thought to be mainly responsible for determining the 'physiological' number of intracutaneous MACs. However, it is as yet unknown whether self-renewing trMAC also exist in human skin. Therefore, we asked in organ-cultured full-thickness human skin, whether human skin also harbors trMAC precursors. Since the pro-inflammatory neuropeptide, substance P (SP), can increase the skin MAC number in mice, a surrogate 'neurogenic inflammation' was induced by SP administration to human skin under serum-free organ culture conditions.

This showed that SP treatment significantly increased the number CD68+ trMACs in the absence of perfused vasculature or bone marrow. Since almost no CD68+/ Ki-67+, CD68+/PH3+ or EdU+/CD68+ cells were detectable in test or control skin, self-renewal of trMAC from proliferating mature intracutaneous MACs is highly unlikely to have accounted for the numeric increase of CD68+ cells. Moreover, SP did not suppress MAC apoptosis, as shown by CD68/TUNEL double-immunostaining. We are currently attempting to exclude the possibility that these trMAC may have been arisen emigrating monocytes still present in cutaneous blood vessels, e.g. by running skin organ culture in the presence of anti-alpha-6 integrin ab, which inhibit monocyte extravasation. However, the fact that intraluminal CD14+ monocytes are found only extremely rarely in either freshly harvested or organ-cultured human skin further supports the hypothesis that, like in mice, trMACs in human skin derive from progenitors seeded in the tissue. Interestingly, CD34+ cells (but not c-Kit+ cells) were often seen to be in direct cell-cell-contact with CD68+ cells in human skin, and since this phenomenon was even more prominent in SP-treated samples, we are currently probing the hypothesis that human skin trMACs arise from tissue-resident CD34+ cells or collaborate with them during trMACs differentiation.

This assay offers a new tool for interrogating human skin MAC biology ex vivo and raises the intriguing question how many of the altered, persisting, pro-inflammatory MACs seen in neurogenic skin inflammation (e.g. atopic dermatitis) actually arise from trMAC, rather than from infiltrating monocytes. So far, our findings suggest that human skin indeed harbors trMACs progenitor cells, whose differentiation into mature MACs can be triggered by SP-driven neurogenic inflammation.

P153

Examining virus-recognizing receptors in Langerhans cells following human skin barrier disruption and stimulation with synthetic RNA

P. Tajpara, P. Kienzl, M. Gschwandtner, C. Schuster, W. Bauer, B. Reininger, M. Mildner and A. Elbe-Bürger *Medical university of Vienna, Dermetology, 1090 Vienna, Austria*

Classic epitheliotropic viruses are able to infect both Langerhans cells (LCs) and keratinocytes (KCs). However, the expression and function of virus-sensing receptors in LCs is still not fully understood. Poly(I:C) is a synthetic analogue of viral double-stranded RNA, which occurs as an important metabolite during viral infection. It is internalized into cells through endocytosis and activates the endosomal TLR3 (Toll-like receptor 3) as well as the cytoplasmic receptors MDA5 (melanoma differentiation-associated gene5) and PKR (protein kinase R). We found that rhodamine-labeled poly (I:C) was rapidly taken up by freshly isolated, FACSsorted human LCs and KCs and induced the production of the proinflammatory cytokine IL-6 in KCs with the same potency as unlabeled poly(I: C). To test whether poly(I:C) is able to induce LC maturation in situ, we applied it topically onto barrierdisrupted full-thickness human skin explants *in vitro*. Twenty four hours after poly(I:C) treatment CD83 and CD86 expression was significantly induced on LCs. Analysis of PRRs recognizing double-stranded RNA in untreated and poly(I:C) treated skin explants, revealed a high baseline expression of TLR3 and PKR in KCs and a weak MDA5 expression exclusively in LCs. In addition, all three receptors were further upregulated by poly(I:C) treatment in the respective cell types. Our data suggest that MDA5 but not TLR3 and PKR may play a key role in the innate immune response of LCs to viral infections.

P154 (O05/04)

NFATc1 promotes imiquimod-induced skin inflammation by negatively controlling IL-10 production in B cells

A. Kerstan[1], H. Alrefai[1,6], K. Muhammad[2], R. Rudolf[2], D. A. Pham[2], S. Klein-Hessling[2], A. K. Patra[2], A. Avots[2], V. Bukur[3], U. Sahin[3,4], S. Tenzer[5], M. Goebeler[1] and E. Serfling[2] *[1]University Hospital Wuerzburg, Department of Dermatology, Venereology and Allergology, 97080 Wuerzburg, Germany; [2]Institute of Pathology, University of Wuerzburg, Department of Molecular Pathology, 97080 Wuerzburg, Germany; [3]Johannes-Gutenberg-University Medical Center, TRON gGmbH-Translational Oncology, Mainz, Germany; [4]Johannes-Gutenberg-University Medical Center gGmbH, Mainz, Germany; [5]Johannes-Gutenberg-University Medical Center gGmbH, Institute for Immunology, Mainz, Germany; [6]Mansoura University, Department of Medical Biochemistry, Mansoura, Egypt*

Background: The repeated topical application of Aldara® cream containing the Tolllike receptor 7 agonist imiquimod (IMQ) to mice triggers potent skin inflammation that displays many aspects of human psoriasis. The cellular interplay of psoriasis is complex and involves e.g. keratinocytes, dendritic cells, T cells, macrophages and mast cells. Due to the rareness of B cells in the inflammatory reaction their role in psoriasis remained largely unappreciated. Most recently, however, it has been shown that IL-10 producing regulatory B cells (B10) regulate IMQ-induced skin inflammation.
Objective: To assess the molecular mechanisms by which B10 cells control IMQinduced skin inflammation.
Materials and methods: Aldara® cream was repetitively applied to the back of wild-type (wt) and B cell-deficient mice. The role of B10 cells in modulating skin inflammation was investigated in mice bearing IL-10-deficient B cells. Furthermore, inflammatory responses were investigated in mice with abrogated expression of nuclear factor of activated T cells c1 (NFATc1) in B cells and mice with IL-10/ NFATc1 double-deficient B cells. IMQ-induced skin inflammation was monitored by the modified PASI (mPASI) score, histologic evaluation and phenotypical as well as functional characterisation of B and T cell populations. Last, chromatin immunoprecipitation (ChIP) and mass spectrometry (MS)-based assays were used for studying potential NFATc1-binding to the IL10 gene including associated proteins.
Results: Evaluation of the mPASI revealed that the repetitive epicutaneous application of IMQ provoked in mice lacking B cells or devoid of IL-10-producing B cells a more pronounced skin inflammation as compared to wt mice. In contrast, ablation of NFATc1 in B cells resulted in a considerable amelioration of mPASI. However, this phenotype was abrogated if IL-10 expression was additionally deleted in NFAT-deficient B cells (IL-10/NFATc1 double-negative B cells). Compared with wt mice the protective effect of NFATc1-deficient B cells on IMQ-induced skin inflammation was accompanied by an enhanced proliferation and IL-10 expression of B cells together with reduced frequencies of CD4+ T cells producing TNF-α, IL-17 and IFN-γ. In co-culture such B10 cells reduced the production of CD4+ T cell cytokines, most prominently TNF-α. On the molecular level, ChIP and MS assays revealed that NFATc1 binds to the IL10 gene and recruits histone deacetylase 1 (HDAC1) thereby suppressing the IL10 gene as shown by consecutive transcriptome analysis of a B cell line overexpressing NFATc1.
Conclusions: IMQ-induced skin inflammation is exaggerated by NFATc1 via a negative regulation of B10 cell activity. Therefore, the modulation of NFATc1 activity in B cells might pave the way for future therapeutic interventions in psoriasis.

P155

Generation of IL-9-producing T cells from healthy human skin explant cultures

P. Kienzl, R. Polacek, T. Gassner, P. Tajpara and A. Elbe-Bürger *Laboratory of Cellular and Molecular Immunobiology of the Skin, Division of Immunology, Allergy and Infectious Diseases, Department of Dermatology, Medical University of Vienna, 1090 Vienna, Austria*

Th9 cells are a recently characterized subset of T cells which play an important role in several diseases including atopic and other inflammatory skin diseases and are rare in healthy human skin. We employed a well-established skin explant culture method that allowed us to investigate the effects of an IL-9 skewing milieu on skin resident T cells. For this purpose, skin biopsies from healthy donors 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 CD3+ T cells (90–98%). Standard conditions yielded more T cells (1.2×10^6/grid) as compared to Th9-promoting conditions (0.8x10^6/grid). In line with this, we found significantly more Ki-67+ T cells at standard conditions as compared to Th9-promoting conditions indicating that cell division contributes to overall cell counts. Interestingly, more CD4+ T cells could be identified in cultures at Th9-promoting conditions (40.8%) compared to standard conditions (24.3%) after 4 weeks. Conversely, more CD8+ cells were present at standard conditions (55.2%) than at Th9-promoting conditions (46.7%). IL-9-producing T cells emerged at week 2 (6%) and increased until week 5 (27%) when cultured at Th9-promoting conditions. In contrast, no IL-9-producing T cells could be identified at week 5 when cells were cultured at standard conditions. Furthermore, CLA could be readily identified on T cells cultured on grids compared to those without grids independent of the medium used indicating that grids were essential to retain CLA on T cells. Together, we could show that IL-2, IL-4 and TGF-beta promote the development of IL-9-producing T cells from healthy human skin. Their exact origin in our culture system is currently under investigation.

P156

Peripheral blood basophil reactivity and frequency are associated with different scores of disease activity in patients with chronic idiopathic urticaria (CIU)

M. M. Rauber, C. Möbs and W. Pfützner *Philipps University Marburg, Clinical & Experimental Allergology, Department of Dermatology and Allergology, 35043 Marburg, Germany*

Background: Urticaria is one of the most common skin diseases with an approximate point prevalence of 0.5–1%. Patients with chronic idiopathic urticaria (CIU) suffer from recurrent pruritus and wheals +/- angioedema for several weeks up to years, substantially affecting their quality of life. However, the pathogenesis of CIU is not fully understood. Almost 35–40% of patients reveal IgG autoantibodies against IgE or its high affinity receptor (FcεRI), suggesting autoimmune mechanisms as one potential pathogenic factor. To get a better understanding of the pathogenesis of CIU we analyzed different immune parameters of patients' peripheral blood basophils (reactivity to different stimuli, binding of immunoglobulins and expression of their receptors on the surface) and the activation of control basophils by patients' serum in relation to their clinical symptoms.
Methods: Peripheral blood was drawn from patients with CIU ($n = 25$) and their urticaria-related symptoms were recorded by the urticaria control test (UCT) and the chronic urticaria quality of life score (CU-Q2oL). The concentration-dependent reactivity of patients' basophils to anti-FcεRI and fMLP as an unspecific control stimulus was evaluated by basophil activation test. Furthermore, IgE and IgG bound by patients' peripheral blood basophils and expression of their respective receptors on basophils' surface were determined by flow cytometry. Finally, basophils of a donor without CIU were incubated with serum of CIU patients followed by flow cytometric measurement of the basophil activation marker CD63. All immunological parameters were compared to patients with allergic rhinitis (AR; $n = 10$) and healthy control subjects (HC; $n = 10$).
Results: CIU patients could be grouped in responder (CIU-R) and non-responder (CIU-NR) depending on basophil reactivity after stimulation with anti-FcεRI. The latter one encompassed a non-reactive cohort (CIU-NR1) and a cohort with high, already pre-existing background activation of basophils, which was further characterized by a basopenic phenotype (<0.1% of basophils; CIU-NR2). Among CIU-NR2 patients basophils showed reduced binding of IgE and IgG antibodies as well as diminished expression of immunoglobulin receptors on their surface in comparison to the other CIU groups, AR patients and HC. Stimulation of non-CIU donor basophils with sera of the different CIU patient cohorts revealed activation of basophils by around 20% of the tested sera, with most sera coming from the CIU-NR2 cohort. Interestingly, these patients showed also markedly pronounced disease severity assessed by UCT and CU-Q2oL compared to the other CIU subjects.
Conclusion: CIU patients can be divided into three distinct subpopulations based on activation of basophils after anti-FcεRI stimulation and peripheral blood basophil numbers. Since the groups differ in the scores of their disease activity, these findings might be of pathophysiological relevance for the severity of CIU.

P157

Impact of test conditions on the activity of antimicrobial peptides

T. Siebert and J. Harder *Department of Dermatology, University of Kiel, 24105 Kiel*

Antimicrobial peptides (AMPs) are effector molecules of the cutaneous innate defense system. They are released by keratinocytes and they are characterized by their capacity to rapidly kill a broad spectrum of microorganisms. Killing assays of AMPs are not standardized and there is increasing evidence that the killing activity of AMPs depends on various factors such as buffer composition and bacteria number. To gain further inside into potential parameters that influence AMP killing activity we tested the activity of two different kinds of important skin-derived AMPs, human beta-defensin-2 (hBD-2) and the antimicrobial ribonuclease RNase 7. We tested their activity against Staphylococcus (S.) aureus, one of the principal skin-associated bacterial pathogen.

First, we tested the activity of these AMPs under typical AMP-assay conditions using 10 mM sodium phosphate buffer (pH 7.2) containing 1% trypticase soy broth (TSB, a typical bacterial growth medium). Bacteria were incubated with various concentrations of AMPs for 3 h and colony forming units (CFU) were determined by plating serial dilutions on agar plates and counting the CFU the following day. These assay conditions confirmed the potent killing activity of the AMPs in the low micromolar range. Interestingly, gradually increasing the TSB concentration decreased the antimicrobial activity and concentrations of 4–6% TSB led to a significant reduction of antimicrobial activity. This explains why we failed to establish conventional MIC (minimal inhibitory concentration) assays with these AMP because in these MIC assays the test substances were incubated in full bacterial growth medium which completely abrogated the activity of the AMP. It is known that increasing salt concentrations inhibit the activity of hBD-2. However, high salt concentrations are unlikely responsible for the observed AMP inhibition because RNase 7 was still active in sodium phosphate buffer (PBS) and 6% TSB contains only low salt concentrations below the salt concentrations reported to inhibit hBD-2 activity. It remains to be shown whether other factors of the TSB (such as anionic peptides which may bind to the cationic AMPs) may be responsible for the interference with the AMP activity. Alternatively, the higher growth rate of bacteria associated with higher TSB concentrations may negatively influence AMP killing activity.

We also tested the influence of different amounts of bacteria in the antimicrobial assay. Increasing the bacteria inoculum 10- to 100-fold did not decrease the relative antimicrobial activity of the AMP. However, the absolute count of surviving bacteria was higher when more bacteria were used in the assay.

Long term storage of AMP under atmospheric conditions may lead to oxidation. We found that RNase 7 is sensitive to oxidation processes and tested the antimicrobial activity of oxidized RNase 7. Oxidized RNase 7 was less active towards S. aureus as compared with the non-oxidized form indicating that oxidation of RNase 7 (and probably also other AMP) may negatively influence their antimicrobial activity.

In summary, our investigations highlight the importance of the assay conditions when evaluating the antimicrobial activity of AMP. In addition, oxidation status of AMP should also be tested before using AMP in antimicrobial assays.

P158

B7-H1 (PD-L1) in the suppressive activity of regulatory B cells (Breg)

M. Schiller, S. Ring, A. H. Enk and K. Mahnke *University Hospital Heidelberg, Department of Dermatology, 69120 Heidelberg, Germany*

B cells are established as antigen presenting and antibody producing cells that have pro-inflammatory functions in several disease settings. But similar to regulatory T cells, also rather immunosuppressive phenotypes were defined recently. The markers for regulatory B cells (Bregs) are manifold. However, the originally described Bregs are characterized by CD19, CD1d, CD5 expression and by production of IL-10. As a novel marker we and others recently found expression of the co-inhibitory molecule B7-H1 (PD-L1) on a subset of Bregs, which may act immunoinhibitory. In order to characterize this novel subset, we studied B cells in spleens, lymph nodes and in peritoneal fluid by flow cytometry. We found that peritoneal fluid contained up to 44% B7-H1 high B cells, whereas in spleens only 13% – and in lymph nodes only 1.5% of the B cells were B7-H1 high. As further key factor to identify Bregs, we analyzed production of IL-10 and found, that 48% of the peritoneal B cells, but only 4% of spleen B cells expressed IL-10 after classical induction by PMA/IONO/LPS. Therefore we concluded that the peritoneal fluid may act as a reservoir for immunosuppressive Bregs. To assess the suppressive function of Bregs we developed an inhibition assay, adding graded doses of Bregs to cocultures of Ovalbumin-pulsed bone marrow derived dendritic cells (DC) and OTII CD4 T cells. T cell proliferation was measured 4 days later. Analysis revealed that PMA/IONO/ LPS activated Bregs were able to suppress T cell proliferation as expected, but notably, when anti-B7-H1 antibodies were applied to the cultures, suppression ceased. Moreover, B7-H1 expression was not only induced by PMA/IONO/LPS but also during culture with antigen loaded DC and T cells. Thus, these data indicate that expression of B7-H1 by B cells, in addition to IL-10 production, is a novel suppressive mechanism that can induce bystander suppression of Breg during cognate DC – T cell interactions.

P159

Cellular stress responses in allergic contact dermatitis

F. Gendrisch[1,2], L. Voelkel[1,2], S. F. Martin[1] and P. R. Esser[1] *[1]University Medical Center Freiburg, Dermatology, Allergy Research Group, 79104 Freiburg, Germany; [2]Faculty of Biology, 79104 Freiburg, Germany*

Contact dermatitis is a T cell mediated skin disease with a high socio-economic impact. While both irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD) result in an eczematous skin reaction, only ACD is mediated by the activation of contact allergen specific T cells.

Although in recent years the importance of innate immune responses for the initial phase of ACD became more and more apparent, knowledge regarding the mechanisms that initiate the skin inflammation remains sparse. We have previously shown that for the full maturation of DCs the activation of innate immune receptors like Toll-like receptor 4 by binding of extracellular matrix derived danger signals in combination with the production of reactive oxygen species as well as the release of ATP and activation of the NLRP3 inflammasome are crucially involved. Several signaling pathways need to be activated in an orchestrated manner – failure of one of these signals results in abrogation of T cell activation. Common to the generation of these danger signals is the induction of tissue stress and damage by contact sensitizers in the skin. Therefore, we now addressed the impact of sensitizers and irritants on the induction of cellular stress responses in the skin and demonstrate that modulation of these stress responses interferes with the induction of ACD in the murine contact hypersensitivity (CHS) model. Interestingly, while abrogation of these stress signals inhibits CHS, their enhancement aggravates the CHS response. Future studies will have to show in a larger scale whether or not the difference between weak and strong contact sensitizers is based on differences in their potency to activate the stress signaling pathways.

Taken together, this underlines our hypothesis that the induction of a proinflammatory milieu within the skin is a crucial pre-requisite for the sensitization and thus provides not only new starting points for the development of causative treatments but also provides a mechanistic explanation for the necessity of the irritant effect of contact sensitizers.

P160

The antimycotic agent clotrimazole inhibits TPA-induced ear swelling in mice by modulating pro-inflammatory cytokine production

O. Brandt[1,2], D. S. Spazierer[1], C. Schuster[1], L. El-Housseiny[1], K. Steinhorst[3], G. Stingl[1] and M. Mildner[1] *[1]Division of Immundermatology and Infectious Diseases, Department of Dermatology, 1090 Vienna, Austria; [2]University Hospital Basel, Department of Dermatology, 4031 Basel, Switzerland; [3]University of Frankfurt, Department of Dermatology, 60590 Frankfurt, Germany*

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

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

We aimed to investigate the anti-inflammatory potency of CLT in two acute inflammation models. Therefore, ear swelling was induced in female BALB/c mice by topical application of either 12-O-tetradecanoylphorbol acetate (TPA) or the contact allergen oxazolone (OXA) following prior sensitization to this agent. 15 min. thereafter, CLT in various concentrations was applied and the resulting ear edema was quantified by measuring the increase of ear thickness at different time points.

While CLT dose-dependently and significantly reduced the TPA-induced ear swelling, no such effect could be observed when OXA-induced ear inflammation was treated with CLT. Accordingly, our observations suggest that CLT selectively suppresses inflammatory pathways that may also be relevant for the concomitant inflammation commonly seen in fungal skin infections.

P161 (O01/05)

Multiple hit immunotherapy of melanoma by generation of CD8+ T cells expressing two additional receptors (TETARs)

U. Uslu, G. Schuler, J. Dörrie and N. Schaft *Universitätsklinikum Erlangen, Dermatology, 91054 Erlangen, Germany*

Introduction: Adoptive T-cell therapy of cancer often fails due to the tumor cells' immune escape mechanisms, like antigen loss or down-regulation and defects in the antigen processing and the MHC presentation machinery. To anticipate these immune escape mechanisms, it would be advantageous to equip T cells with multiple specificities and MHC-independent receptors.

Methods: To study the possible interference of a T-cell receptor (TCR) with a chimeric antigen receptor (CAR) after transfer into one T cell, and to examine how to counteract possible competing effects, we generated TETARs, CD8+ T cells expressing two additional receptors by simultaneous transfection with a TCR and a CAR using RNA electroporation. The TETARs were equipped with a TCR specific for the common melanoma antigen glycoprotein 100 (gp100) and a CAR recognizing the melanoma surface antigen melanoma-associated chondroitin sulfate proteoglycan (MCSP). To prevent competitive effects between both introduced receptors, the quantities of TCR/CAR-encoding mRNAs were titrated to obtain suitable ratios for transfection.

Results: Cell surface staining of transfected CD8+ T cells showed that TETARs can be generated by simultaneous transfection of receptor-encoding mRNAs using electroporation. The transfection with different ratios of RNA encoding the gp100 TCR and the MCSP CAR revealed a ratio of 5 μg gp100 TCR RNA and 10 μg MCSP CAR RNA to be the most suitable combination for generating TETARs considering expression level of both. Regarding the expression kinetics, the CARs seemed to reach the peak of expression already 6 h after electroporation, which was maintained until the latest investigated time-point of 24 h. The expression of the TCRs increased slowly but continuously until the last measured time-point of 24 h. Regarding functionality, antigen-specific cytokine secretion efficiency of TETARs was similar to – or even better than – that of T cells transfected with a single TCR or a single CAR. Also, TETARs were able to lyse target cells antigen specifically as good as T cells transfected with a single TCR or a single CAR. The transfection of TETARs with different ratios of RNA encoding the gp100 TCR and the MCSP CAR revealed that the cytolytic capacity of all used ratios was satisfactory. Regarding the cytolytic kinetics of transfected T cells, the cytolytic capacity of TETARs transfected with different quantities of TCR/CAR-encoding mRNAs was similar 18 h and 40 h after electroporation of the T cells. Further investigations will be performed to analyze the cytolytic capacity of the TETARs at later time points after transfection of the T cells. Also, we want to prove that TETARs can be activated more efficiently than a mixture of 2 pools of CD8+ T cells each reprogrammed with only one specificity.

Conclusions: Taken together, we generated dual-specific CD8+ T cells directed against the common melanoma antigens gp100 and MCSP for the use in adoptive T-cell therapy of melanoma. These TETARs proved functional in cytokine secretion and cytolytic activity upon stimulation with each of their cognate antigens. No reciprocal inhibition was observed. As the generation of TETARs helps by-passing major mechanisms by which tumors escape immune recognition, this option may open up new avenues in immunotherapy of melanoma.

J.D. and N.S. share senior authorship.

P162

Effects of antipsoriatic therapies on keratinocytes biology and immune cells

J. Holstein, J. Brück, B. Fehrenbacher, A. S. Yazdi, F. C. Eberle and K. Ghoreschi *Eberhard Karls University Tuebingen, Department of Dermatology, Tuebingen, Germany*

Psoriasis is a chronic inflammatory skin disease with aberrant keratinocyte proliferation. The disease manifestation is associated with the expression of innate cytokines and adaptive immune responses orchestrated by interleukin (IL-)17- producing CD4+ T cells (Th17 cells). In line with this, modern targeted therapies with biologics neutralizing innate cytokines like TNF or cytokines involved in the Th17 pathway (IL-23/IL-17) are highly effective in psoriasis. However, traditional antipsoriatic therapies with topical anthralin improve psoriasis and clear the skin disease even more rapidly. While modern biologics have selective mode of actions, the psoriasis-improving effects of anthralin on keratinocytes and immune cells are not fully understood. Here we studied the effects of anthralin on psoriatic skin, especially on keratinocyte biology and on Th17 cells.

In our first experiments, we performed skin histology from lesional psoriatic skin before and during early phase of therapy. We found a significant reduction in epidermal thickness and keratinocyte proliferation as determined by Ki67 staining. Further, we studied keratinocyte differentiation of skin samples by immunofluorescence staining of keratins 5, 10 and 16 and found significant changes after initial treatment. In addition, we analyzed changes in the infiltration of psoriatic plaques by Th17 cells before and during treatment by performing antibody stainings of skin sections for CD4 and IL-17 for immunofluorescence. We found a reduction in the number of CD4+IL-17+ Th17 cells during treatment. Finally, we performed gene expression analysis of patient's skin biopsies before and during initial treatment and compared all results with biopsies from patients treated with modern biologics. Our results show that anthralin affects keratinocyte biology, Th17 cells and cytokine expression in a different mode than neutralizing anti-cytokine antibodies. The mechanistic pathways are under current investigation.

P163

Reduction of hypersensitivity type IV induced skin inflammation by high dose antigen sensitization

S. Klein, J. Westermann and K. Kalies *Institute of Anatomy, 23562 Luebeck, Deutschland*

Delayed type of hypersensitivity (DTH; allergy type IV) is a CD4+ Th1 cell mediated antigen-specific skin inflammation induced by skin exposure to an antigen after sensitization. Antigens can be small molecules such as haptens, nickel or formaldehyde but also large proteins and pathogens such as heterologous blood cells or Leishmania major parasites. For the latter antigens it has been shown that increasing the antigen dose for sensitization leads to suppression of the DTH response. The mechanism behind this antigen-dose dependent suppression of the DTH response is unknown and could be a therapeutic option in disease condition such as contact dermatitis. To find out we studied the dose dependent response to Sheep Red Blood Cells (SRBC) in the spleen of mice after intravenous sensitization. Our data show that a high antigen dose leads to an activation of B cells in the spleen, which we could identify as the critical important event for suppression of DTH. Consequently, high dose sensitization cannot suppress the DTH response in B cell deficient mice.

To further identify the mechanism behind this suppression of the DTH response we used C5aR-KO mice and CD40L-KO mice. C5aR-KO mice were used since it has been shown that the lack of C5aR leads to a decreased expression of MHC-II on B cells and therefore serve as model for an impaired antigen presentation. CD40L-KO mice were used since the lack of CD40L abolishes the interaction between activated antigen-specific T cells and their cognate B cells completely.

Our results show that a decreased antigen presentation altered the reaction in the spleen (day 3 after sensitization), however the DTH response was unaffected. This leads to the conclusion that the lowered antigen presentation in C5aR-KO mice is only of minor importance for suppression of the DTH response.

In contrast, CD40L-KO showed an unexpected effect in complete blockage of the DTH response even though a strong T cell response was found in the spleen. This indicates that either (i) the interaction between T and B cells via CD40-CD40L in the spleen is needed for activation of skin homing T cells or (ii) the CD40-CD40L interaction in the skin between T cells and other antigen presenting cells is of critical importance for DTH development. This data indicate that the CD40-CD40L interaction might be a promising target for therapeutic intervention, which clearly needs further investigation. In addition, it will be important to find out in further studies whether application of a high antigen dose can be used to suppress the DTH response in the skin even if applied after sensitization with a low dose.

P164

Neutrophil granulocyte counts correlate to PASI-response under TNF-α antagonist treatment

J. Hoffmann, C. Knoop, A. H. Enk and E. N. Hadaschik *Universität Heidelberg, Hautklinik, 69121 Heidelberg, Germany*

TNF-α inhibitors Adalimumab and Etanercept, and IL12/IL23 antagonist Ustekinumab are used to treat psoriasis and psoriasis-arthritis. General alterations of inflammatory biomarkers under treatment with these biologics have been reported, a detailed description of parameter dynamics and a correlation to treatment response is, however, lacking. Here, we present a retrospective data analysis of 113 patients with psoriasis receiving Adalimumab, 64 psoriasis patients receiving Etanercept, and 23 patients receiving Ustekinumab. Anti-nuclear antibody (ANA) titers, antidoublestranded- DNA (anti-dsDNA) concentrations, polymorphonuclear cell (PMN) counts, Non-PMN leucocyte counts, C-reactive protein (CRP) concentrations and PASI values at baseline and during treatment were recovered. ANA-titers and antidsDNA concentrations significantly increased under treatment with. PMN counts considerably decreased under treatment with Adalimumab and Etanercept and, to a lesser extent, under treatment with Ustekinumab. Interestingly, statistical analysis using generalized estimating equations revealed a positive association of PASI-values and neutrophil counts, but not Non-PMN leucocyte counts, in patients treated with Adalimumab and, to a lesser extent, Etanercept, that was independent of treatment duration and CRP. The role of PMN in psoriasis pathogenesis has recently received much attention. Histologically, numbers of PMN in psoriatic lesions vary depending on disease state and individual patient. The present data illustrates differential effects of biologics on inflammatory biomarkers, particularly PMN counts, and supports the role of neutrophil granulocytes as relevant targets of immunosuppressive treatments in psoriasis. One may speculate that these effects contribute to the differential therapeutic efficacy of treatments observed in individual patients.

P165

Frequency of plasmacytoid dendritic cells (pDCs), myeloid-derived suppressor cells (MDSCs) and T regulatory cells (Tregs) in peripheral blood of patient with psoriasis

J. Wegner, F. K. Krebs, A. Tuettenberg and E. von Stebut *University Medical Center, Department of Dermatology, 55131 Mainz, Germany*

Psoriasis is one of the most common chronic T cell-mediated inflammatory skin and joint diseases in humans with a prevalence of approximately 2% worldwide. Severe manifestations of the disease induce increased patient morbidity. It is known that keratinocytes and lymphocytes play an important role in the pathogenesis of psoriasis, but little is known about the significance of pDCs, MDSCs and Tregs – all of which exhibit regulatory functions of immune responses – in this respect. The goal of our study was to analyze and determine number and function of bloodderived pDCs, MDSCs and their interaction with Tregs in psoriasis. To this end, we performed a comparative analysis of the frequency of these three circulating regulatory cell populations in psoriatic patients and in healthy donors. We assessed these cells in the peripheral blood of 12 psoriatic patients who did not receive any systemic therapy, but differed in disease severity. To measure the severity and extent of psoriasis, we used the PASI score (Psoriasis Area and Severity Index). In our patient cohort, the score range was between 4.3 and 45.8 points. Three healthy donors served as control group. For pDCs, the mean percentage of viable CD3- CD19-CD303+ and CD3-CD19-CD304+ pDCs was higher in psoriatic patients as compared to healthy donors, while the value of CD3-CD19-CD123+ was comparable for both groups. In addition, for MDSCs, the mean percentage of CD14+CD11b +CD33+HLA-DRlow was clearly increased in psoriatic patients, whereas the levels of CD14+CD11b+CD33+HLA-DRlowCD15hi and CD14+CD11b+CD33+HLADRlowCD124hi cells remained unaltered in psoriatic compared to healthy patients. Interestingly, for Tregs, the mean percentage of CD3+CD4+CD25+Foxp3+CD127hi, CD3+CD4+CD25+Foxp3+HLA-DRhi, CD3+CD4+CD25+Foxp3+GARPhi, CD3+CD4+CD25+HLA-DRhiGARPhi and CD3+CD4+CD25+HLA-DR+CD127hi cells were increased in all patients with psoriasis compared to controls. In summary, our results indicate differences in the frequencies of pDCs, MDSCs and Tregs in peripheral blood of psoriatic patients compared to healthy donors. To verify these findings, we intend to analyze blood of larger patient cohorts and plan to control for the effect of disease severity and impact of systemic therapy on the respective marker expression. These studies will aid a better understanding of regulatory mechanisms contributing to control of psoriasis activity.

P166

Evaluation of the effect of TNT003, a classical pathway specific inhibitor, onbullous pemphigoid sera induced complement deposition on human skin

A. Kasprick[1], S. Panicker[2], C. Kauderer[1], F. Petersen[3], E. Schmidt[1] and R. J. Ludwig[1] *[1]Luebeck Institute of Experimental Dermatology, University of Luebeck, Luebeck, Germany; [2]True North Therapeutics, Inc., San Francisco, USA; [3]Priority Area Asthma and Allergy, Research Center Borstel, Borstel, Germany*

Bullous pemphigoid (BP) is the most prevalent autoimmune blistering disease in Europe and the USA and is clinically characterized by subepidermal (muco)- cutaneous blistering and intense pruritus. Caused by autoantibodies against type XVII collagen, BP also features activation of the classical complement pathway and induction of an inflammatory milieu and infiltration of inflammatory cells in the skin. Despite increasing insights into its pathogenesis, treatment of BP involves the long term application of superpotent topical or systemic corticosteroids and as such is associated with severe adverse events and a high relapse rate of 30–50% within a few months after withdrawal of the drug. Consequently, there is a pronounced medical need to develop novel treatment options. A potential therapeutic target for BP treatment could be inhibition of complement activation. Here, we evaluated the effect of TNT003, a mouse monoclonal antibody that inhibits the classical pathway specific serine protease C1s, on BP immune complex mediated complement activation by the use of an indirect complement activation assay.

Therefore, human skin sections were incubated in the presence of BP sera to form immune complexes between patient BP autoantibodies and collagen XVII at the dermal-epidermal junction (DEJ). Following the addition of an exogenous complement source (normal human serum), complement activation was assessed by examining the deposition of C3 fragments at the DEJ via indirect immunofluorescence (IF) microscopy.

Of the 91 individual BP sera samples tested, 32 were able to fix detectable amounts of complement fragment C3c and, consequently, were included in this study. When performing the assay in the presence of TNT003, IF staining revealed diminishing deposition of complement fragment C3c at the DEJ in a concentration-dependent manner. Furthermore, complement activation was also measured by the amount of generated anaphylatoxins C3a, C4a, and C5a in assay supernatants. While we did not observe any further anaphylatoxin production in skin sections incubated with BP patient sera compared with healthy control sera, TNT003 significantly reduced C4a and C5a formation while production of anaphylatoxin C3a remained unaffected. Furthermore, the chemotactic activity of supernatants on neutrophils was diminished in samples with reduced anaphylatoxin formation due to TNT003 treatment. However, blocking complement had no effect on the release of reactive oxygen species by neutrophils.

Taken together, these data provide evidence that complement activation induced by BP immune complexes can be inhibited *in vitro* by TNT003. Thus, a good safety record provided, a humanized version of TNT003 may prove to be an efficacious therapeutic alternative to corticosteroids in BP patients.

P167

Functional consequences of myeloid cell-specific Stat3 activation in skin fibrosis

N. Do, S. Willenborg, X. Ding and S. A. Eming *University of Cologne, Dermatology, 50937 Cologne, Germany*

Skin wound healing is characterized by the replacement of granulation tissue with extracellular matrix. Pathological healing conditions, as associated with chronic venous diseases, diabetes mellitus or autoimmunity, often cause excessive accumulation of fibrous connective tissue leading to fibrosis and organ malfunction. Inflammation is considered a key factor driving the development of progressive fibrotic diseases. However, detailed understanding how elements of the inflammatory cascade might induce and sustain a fibrotic response is elusive. In this study we aim to unravel the functional impact of macrophage polarization during the development of skin fibrosis. Here we investigated myeloid-cell restricted signaling of Stat3, a transcription factor implicated in the resolution of inflammatory responses.

To unravel the functional impact of macrophage polarization during the development of skin fibrosis, we generated myeloid cell-specific Stat3 deficient mice (STAT3-MKO) and investigated bleomycin-induced skin fibrosis in STAT3-MKO mice. In STAT3- MKO mice the fibrotic response was significantly increased after 2 weeks of daily bleomycin challenge when compared to control mice. Accelerated development of skin fibrosis in STAT3-MKO mice was characterized by a significantly altered macrophage activation phenotype when compared to controls. Whereas FACSsorted macrophages from fibrotic tissues in control mice revealed a robust induction of mediators that have been associated with anti-fibrotic activities such as IL-10, decorin, SOCS3, IL-1β and TIMP-1, expression of these factors was significantly reduced in macrophages of STAT3-MKO mice.

Based on these *in vivo* data we suggest a Stat3-mediated anti-fibrotic role for macrophages in bleomycin-induced skin fibrosis. Macrophages might regulate the fibrotic activity of fibroblasts via limiting the availability of active TGF-β. Here we focus on two mediators that could regulate TGF-β availability: decorin and SOCS3. Decorin expression, which inhibits binding of TGF-β to its receptor, is attenuated in Stat3-deficient macrophages in fibrotic lesions. Furthermore, SOCS3 regulates TGF-β expression at the transcriptional level. It is known that TGF-β can induce its own expression in macrophages and fibroblasts. However, SOCS3 interrupts TGF-β signaling by binding phosphorylated Smad3 and thus impairs translocation of the transcription factor into the nucleus. Physiologically, IL-10 induces SOCS3 expression via Stat3 which leads to a suppression of TGF-β transcription in control macrophages. However, in the absence of SOCS3 pSmad3 could freely translocate into the nucleus and promote TGF-β signaling and TGF-β expression. So far, we confirmed induction of SOCS3 and suppression of TGF-β in control macrophages upon IL-10 stimulation in an *in vitro* experiment. Consistently, Stat3-deficient macrophages did not express SOCS3 or down-regulate TGF-β upon IL-10 stimulation. In order to further investigate how macrophages regulate the development of fibrosis, we are currently performing macrophage-fibroblast coculture experiments to examine aspects of their crosstalk.

Together, our findings provide new mechanistic insights into macrophage-mediated skin fibrosis which might be relevant for the development of novel anti-inflammatory therapies to prevent tissue fibrosis and scarring.

P168

Self DNA stimulates keratinocytes to produce IFN-regulated chemokines: Implications for the pathogenesis of cutaneous lupus erythematosus

S. Zahn, J. Maier, T. Bieber, T. Tüting and J. Wenzel *University Hospital Bonn, Dermatology and Allergy, Bonn*

Cutaneous lupus erythematosus (CLE) is an autoimmune disease characterized by a strong lesional type I interferon (IFN) associated inflammation. Keratinocytes are known to determine the interface-dermatitis-pattern in CLE by production of proinflammatory cytokines in the lower epidermis, but the mechanisms are largely unknown. We provide evidence for an important role of endogenous nucleic acids in the pathogenesis of CLE.

By using gene expression analysis, we show that an excessive activation of the innate immune system via TLR-dependent and TLR-independent pathways is a hallmark of lesional inflammation in CLE. We demonstrate that keratinocytes produce large amounts of IFN-inducible proinflammatory cytokines mainly in response to stimulation of cytosolic nucleic acid receptors. Immortalized keratinocytes (HaCatcells) as well as normal epidermal keratinocytes (NEHK) produce CXCL9, CXCL10, and IFN-λ in response to the synthetic stimuli polyIC, 3pRNA and polydAdT. Furthermore, we demonstrate that

natural ligands (endogenous nucleic acids) also drive the expression of these chemokines, in cultured keratinocytes as well as in 3Depidermis models (EPICS). Our findings in knockout mice, which lack the cytosolic DNase TREX1 and develop CLE-like skin lesions after UV-stimulation, reveal the capacity of endogenous DNA to stimulate LE skin disease.
Our results provide strong evidence for a functional pathogenetic role of endogenous nucleic acids in CLE. Immunostimulatory nucleic acids, which are released within the cytotoxic inflammation along the dermo-epidermal junction, have the capacity to drive the lesional interface dermatitis and to maintain the inflammatory vicious circle in this autoimmune disease.

P169 (O02/05)

Expression of CD39 by Treg mediates degradation of ATP, affecting shedding of CD62L and regulating contact hypersensitivity reactions

K. Mahnke, S. Ring, A. Pushkarevskaya, M. Schiller and A. H. Enk *University of Heidelberg, Department of Dermatology, 69120 Heidelberg, Germany*

Application of contact sensitizers to the skin can trigger danger signals such as extracellular ATP that activates T cells and promotes sensitization. In a murine TNCB-induced contact hypersensitivity (CHS) model we found that injection of regulatory T-cells (Treg) blocks sensitization against haptens. Very early after sensitization (2 h–3 h) we also noted a transient increase in size and cellularity of the skin draining lymph nodes (LNs). This led us to hypothesize that Treg may affect the trafficking of T cells from and to LNs by modulating LN-homing molecules in lymphocytes. Two to three hours after sensitization we found that fewer CD8+ T cells expressed CD62L in LNs as compared to controls (60% vs. 76%). In contrast, i.v. injection of Treg prevented downregulation of CD62L on CD8+ T cells after sensitization, whereas injections of Treg devoid of CD39 were unable to do so. As for the mechanism of CD62L regulation we found that ATP, which is released in skin upon hapten-exposure, is inducing the protease ADAM17 in LNs via engagement of P2X7-ATP receptors. ADAM17 cleaves CD62L from the surface of LN-residing T cells, which in turn may provide one signal for T cells to leave the LNs. This regulation is disturbed by the presence of Treg, as Treg remove extracellular ATP from the tissue by activity of CD39 and therefore abrogate the shedding of CD62L. In summary these data indicate that the regulation of ATP turnover by Treg in skin is an important modulator for immune responses.

P170

Tumor-homing eosinophils predict the clinical course of malignant melanoma

A. Hufeland[1,2], A. Funder[1,2], C. Kehrel[1,2], R. Lichtenberger[1,2], D. Tichy[3], T. Holland-Letz[3], E. Herpel[4,5], V. Umansky[1,2], J. Utikal[1,2] and C. Gebhardt[1,2] *[1]Deutsches Krebsforschungszentrum (DKFZ), Skin Cancer Unit, 69120 Heidelberg, Germany; [2]University Hospital Mannheim, Department of Dermatology, Venereology and Allergology, Mannheim; [3]Deutsches Krebsforschungszentrum (DKFZ), Biostatistics, Heidelberg; [4]National Center for Tumor Diseases (NCT), Biobank, Heidelberg; [5]University Hospital Heidelberg, Pathology, Heidelberg, Deutschland*

The interaction of the patient's immune system with melanoma impacts on the clinical outcome and might provide important implications for the identification of prognostic markers. The specific immune reaction is represented by immune cell infiltrates.
Here we systematically analyzed the presence and localization of tumorinfiltrating immune cells on tissue-microarrays displaying 59 primary melanoma, 70 corresponding metastases as well as in 41 associated benign nevi and evaluated their clinico-pathological impact and using the Kaplan-Meier method and Cox proportional hazards model, and Mann-Whitney-U or Kruskal-Wallis test. Immune cells were detected using immuno-histochemistry and specific antibodies.
Higher levels of activated eosinophils as well as tumor-infiltrating T lymphocytes, and T-memory cells were significantly associated with longer progression-free (PFS) as well as overall survival (OS) whereas higher levels of tumor-infiltrating neutrophils were significantly associated with shorter PFS and OS.
Eosinophils as well as tumor-infiltrating T cells, memory T cells, and neutrophils are independent prognostic tissue markers that might be central for elucidating the specific immune cells-melanoma cell interaction.

P171

Eosinophils, MDSCs and neutrophils are novel predictive biomarkers in melanoma treatment with checkpoint inhibitors

C. Gebhardt[1,2], A. Sevko[1,2], H. Jiang[1,2], R. Lichtenberger[1,2], M. Reith[1,2], K. Tarnanidis[1,2], L. Umansky[3], P. Beckhove[3], A. Sucker[4], D. Schadendorf[4], J. Utikal[1,2] and V. Umansky[1,2] *[1]German Cancer Research Center (DKFZ), Skin Cancer Unit, Heidelberg, Deutschland; [2]University Hospital Mannheim, Department of Dermatology, Venereology and Allergology, Heidelberg, Deutschland; [3]German Cancer Research Center (DKFZ), Department of Translational Immunology, Heidelberg, Deutschland; [4]University Hospital Essen, Department of Dermatology, Essen, Deutschland*

Ipilimumab improves the survival of patients with metastatic melanoma. Since only around 20% of patients experience long-term benefit, reliable markers are needed to predict a clinical response. Therefore, we asked whether some myeloid cells and related inflammatory mediators could serve as predictive factors for the patients' response to ipilimumab.
Peripheral blood of 59 stage IV melanoma patients was analyzed before the treatment and at different time points upon the therapy using a clinical laboratory analysis, multi-color flow cytometry as well as ELISA or bio-plex assays.
An improved clinical response was associated with an early increase in eosinophil count during the treatment with ipilimumab. In contrast, in non-responders compared to responders elevated amounts of monocytic myeloid-derived suppressor cells (moMDSCs), neutrophils, and monocytes were found. Upon the first ipilimumab infusion, non-responders displayed elevated serum concentrations of proinflammatory damage-associated-molecular-pattern molecules (DAMP) such as S100A8/A9 and HMGB1 that are known to attract and to activate MDSCs.
These findings shed light on additional mechanisms of ipilimumab effects and provide clinical support for the measurement of immune cell subtypes such as eosinophils, neutrophils, and MDSCs as well as S100A8/A9 and HMGB1 before and during ipilimumab treatment in order to predict a clinical treatment response.

P172 (O06/03)

The RAG recombinase promotes survival and proliferation of dermal innatelike lymphoid cells type 2

M. M. Saleh, K. Honold, M. P. Schön and T. Buhl *University Medical Center Goettingen, Dermatology, Goettingen*

CD103+ dermal Innate-like Lymphoid Cells (dILC2s) are centrally involved in Th2- driven inflammatory diseases such as atopic dermatitis and allergy. Since Rag (recombination activating gene) is needed for the rearrangement and recombination of the genes of immunoglobulin and T cell receptor molecules during V(D)J recombination, most research on ILCs has been carried out in $Rag^{-/-}$ mice due to their lack of T and B cells. Interestingly, it has been reported recently that 30–40% of ILC2s express Rag1 in their development.
We found that Rag1-KO mice have 2–3fold higher total dILC2s number compared to WT BL6/J mice. Total dILC2 numbers in other mouse strains with immune deficiencies (such as $MyD88^{-/-}$, $TLR3^{-/-}$, $CD103^{-/-}$) were unchanged. Although phenotypically similar, contact hypersensitivity (CHS) models with DNFB lead to a dramatic decrease of dILC2s numbers in Rag1-KO mice, whereas these cells significantly increased in WT mice. Adoptive transfer of WT lymphocytes into Rag1- KO mice did not abolish this effect in DNFB CHS. We therefore searched for an intrinsic cause in dILC2, and we found a significantly higher baseline apoptosis rate in $Rag1^{-/-}$ dILC2. Moreover, Rag1-KO dILC2s displayed an impaired proliferation rate. Transcriptome analysis of dILC2 from $Rag1^{-/-}$ and WT revealed significant expression alterations beyond immunoglobulin and T cell receptor molecules.
In summary, our data suggest an important role of Rag proteins in ILC2 cell survival and proliferation beyond the well-known effects of Rag on T and B cell maturation. Previous studies on ILC phenotype and functions in $Rag^{-/-}$ mice need to be carefully re-evaluated.

Infectious Diseases

P173 (O06/01)

Mast cell-derived IL-6 is critical for the healing of infected wounds in mice

C. Zimmermann, D. Troeltzsch, M. Metz, M. Maurer and F. Siebenhaar *Charité – Universitätsmedizin Berlin, Department of Dermatology and Allergy, 10117 Berlin, Germany*

Skin wound infection is a considerable health problem that gains importance due to increasing antibiotic resistance. A better understanding of the innate defense mechanisms against bacterial superinfection could lead to novel treatment approaches. Mast cells (MCs) have been shown to contribute to optimal host defense against bacterial infections. However, the role of MCs in clinically relevant bacterial skin wound infections is poorly understood. We, therefore, established a model of Pseudomonas aeruginosa (PA) skin wound infection in mice and characterized the critical factors involved in antibacterial host defense responses and optimal wound healing. Using mast cell-deficient KitW/KitW-v and Cpa3-Cre; Mcl-1 fl/fl mice, we observed significantly delayed wound closure in PA infected skin wounds in the absence of MCs. We have reported previously, that this delay in wound closure was associated with a 10-fold reduction in bacterial clearance and that engraftment of MCs into the skin of KitW/KitW-v mice restored both, bacterial clearance and wound closure, to wild type levels. Co-culture of MCs and keratinocytes (KCs) infected with PA led to a significant increase of MC-derived IL-6 *in vitro*. Here we demonstrate that local engraftment of MC-deficient KitW/KitW-v mice with IL-6-deficient MCs failed to control PA wound infection and restoration of normal wound healing *in vivo*. Most notably, treatment with recombinant IL-6 induced antimicrobial peptide production by KCs *in vitro* and resulted in the control of PA infection and normal wound healing *in vivo*. Taken together, our results demonstrate for the first time that skin wound infection by PA and the impaired healing of superinfected wounds are controlled by MCs and reveal a novel antimicrobial defense mechanism that requires the release of MC-derived IL-6. These findings offer new strategies for the prevention and treatment of antibiotic resistant bacterial skin wound infections.

P174

Establishment of a real-time-PCR-assay for routine identification of Trichophyton rubrum, Trichophyton interdigitale, Microsporum canis, and Trichophyton species of Arthroderma benhamiae

C. Wiegand, E. Maschke, D. Reichmann and U. Hipler *Universitätsklinikum Jena, Jena*

Introduction: The basis for an effective treatment of a dermatomycosis is the correct and timely identification of the causative agent. This allows for targeted and specific antiepidemic measures. However, conventional identification methods like culture and microscopy are slow and mostly based on morphological characteristics which make them less sensitive and specific. Modern approaches based on molecular biological methods, like real-time-PCR-assays, are compared to that quick as well as accurate and therefore steadily gain acceptance. Our aim was to establish a fast, sensitive, and reliable real-time-PCR-assay for identification of the four dermatophytes Trichophyton rubrum, Trichophyton interdigitale, Microsporum canis, and Arthroderma benhamiae which we commonly find in our daily practice.
Methods: 314 diverse samples from patients (scales, hair, and nail clippings) were analyzed. Dermatophytes were identified using native preparation as well as the characteristic macroscopic and microscopic features of the cultures. Moreover, specimens were subjected to DNA extraction and subsequent real-time-PCR-assay for identification of T. rubrum, T. interdigitale, M. canis, and A. benhamiae.
Results: The results of the real-time-PCR-assay were verified with diagnostic findings by conventional methods to evaluate its reliability, sensitivity, and specificity as well as demonstrate advantages over native preparation and fungal cultures with regard to rapidity. It could be shown that this method is suitable for routine use in our lab. It was found to be reliable and sensitive. Moreover, it was moderate specific, only four infrequent dermatophytes were wrongly recognized. Therefore, further optimization is necessary. Nonetheless, a significant saving of time was demonstrated. A first diagnose of the causative agent is possible 24 h after patient samples have been sent to the lab using the real-time-PCR-assay while sole dependence on conventional methods results in time frames of up to 3 or 4 weeks.
Conclusions: We could establish the real-time-PCR-assay as a molecular biological method for direct identification of dermatophyte DNA in clinical samples. It proved to be a highly sensitive, specific, rapid and reliable tool that is independent of time consuming culture evaluation and biochemical methods. The ability to identify dermatophytes up to species level is a step forward in solving the problems of ensuring that correct therapies are initiated early for these patients.

P175 (O02/01)

A single CD8+-epitope as vaccine against murine cutaneous Leishmaniasis

K. Dietze-Schwonberg[1], S. Brosch[1], G. van Zandbergen[2], B. Grewe[1], H. Schild[3], S. Tenzer[3] and E. von Stebut[1] *[1]Department of Dermatology, University Medical Center, Johannes Gutenberg University, Mainz; [2]Division of Immunology, Paul-Ehrlich-Institute, Langen; [3]Institute of Immunology, University Medical Center, Johannes Gutenberg University, Mainz*

Infection with cutaneous Leishmaniasis is caused by the protozoan Leishmania (L.) major and healing of infection is based on IFNgamma secretion by CD4+ and CD8+ T cells. Up to date, no vaccine against this human pathogen exists. However, it is still unclear which proteins/peptides are needed for parasite clearance and therefor can be used as a vaccine. To now expedite the generation of a vaccine, we identified the most abundant peptides expressed by both life-forms – infectiousstage promastigotes and intracellular amastigotes – by mass spectrometry. Based on their predicted immunoreactivity using a computer-based algorithm (SYFPEITHI), we next choose 300 H2-Db and H2-Kb peptides for further analysis. All 300 peptides were tested in *in vitro* experiments. Peptides were co-cultured with C57BL/6- DC and primed CD8+ T cells for 48 h. Supernatants were assayed for the amounts of secreted IFNgamma, IL-4 and IL-10 by ELISA. Based on their cytokine-profile, we subsequently selected 23 peptides for evaluation of their potential to protect C57BL/6 mice against challenge with L. major. Mice were immunized in a prime/boost/boost approach with 20 μg peptide followed by 2× 10 μg peptide in combination with CpG as adjuvant in one ear. One week later, the mice were infected with physiological low-dose inocula of 1000 metacyclic promastigotes in

the contralateral ear and lesion volumes were measured weekly. Interestingly, one peptide (p54) was able to protect mice against challenge compared to control mice, whereas all other tested peptides failed. Lesion volumes were significantly reduced in p54-immunized mice compared to control mice. Additionally, we analysed local and systemic parasite burdens. In line with reduced lesion volumes, mice immunized with p54 had reduced numbers of parasites in their ears and spleens. Peptide p54 is part of a housekeeping protein and can be found in other Leishmania subspecies as well. To summarize, we identified a MHC class I-restricted peptide which protected C57BL/6 mice against challenge with L. major, thereby providing the basis for establishing a long desired vaccine against this human pathogen.

P176

A protective immune response against pathogens triggered by the skin microbiota is dependent on the integrity of the epithelial barrier

M. S. Burian, B. Kraft and B. Schittek *University Hospital Tuebingen, Department of Dermatology, 72076 Tuebingen, Germany*

Human skin, the primary interface between the body and environment, is constantly exposed to an immense number of potential pathogens, while at the same time allows commensal bacteria to colonize and form a tissue specific microbiota. This skin resident microbiota play an important role in innate and adaptive immune responses against pathogens such as Staphylococcus aureus. Keratinocytes, as the most abundant cell type in the epidermis, actively participate in the innate immune response towards pathogens e.g. by cytokine production or expression of antimicrobial peptides. Skin commensal bacteria are able to amplify the immune response of keratinocytes and create a protective environment by immune conditioning of the epithelial barrier. We show that the skin resident bacterium Staphylococcus epidermidis is not only able to amplify defensive immune responses in human primary keratinocytes, but also have a protective effect on pathogen attachment and invasion. This effect on S. aureus attachment and invasion could be observed using alive S. epidermidis and culture supernatant, indicating that S. epidermidis secreted factors might be responsible for immune conditioning of the epithelial barrier. Furthermore, by using an epicutaneous mouse skin infection model, we demonstrate that skin barrier defects reverse the protective effect of S. epidermidis and increase skin colonization with pathogens. In addition, current experiments using knock-out mice address the molecular mechanisms of the immune-modulating effect of skin commensals on pathogen infection. In summary, we show that skin commensal bacteria are able to amplify the innate immune response against pathogens and create a protective environment by immune conditioning of the epithelial surface *in vitro* and *in vivo*, which is however dependent on the integrity of the epithelial barrier.

P177

Effect of Staphylococci on Tight Junctions – involvement in innate immunity

K. Bäsler[1], M. Galliano[2], P. Houdek[1], B. Guiraud[2], S. Vidal-y-Sy[1], E. Wladykowski[1], H. Rohde[1], S. Bessou-Touya[2], H. Duplan[2] and J. M. Brandner[1] *[1]University Medical Center Hamburg-Eppendorf, Dermatology and Venerology, 20246 Hamburg, Germany; [2]Centre R&D Pierre Fabre, 31035 Toulouse, France*

The skin is a pivotal barrier against the uptake of pathogens and allergens and the skin barrier is part of the innate immune system. Tight Junctions contribute to the overall skin barrier by forming a physical barrier in the stratum granulosum and by influencing the stratum corneum. We have shown previously that skin infection with S. aureus and S. epidermidis result in a short term increase of TJ proteins in the granular cell layer followed by a loss of the proteins. The aim of this study was to investigate the influence of S. aureus and S. epidermidis on TJ functionality and to elucidate the underlying mechanisms.

By using phk we observed a dose dependent increase of transepithelial resistance (TER) and decrease of paracellular permeability for a 4 kDa tracer during short time incubation with S. epidermidis and S. aureus. This argues for a prevention mechanism by strengthening of the innate immune system via tightening of the TJ barrier to reduce/delay pathogen uptake. However, even though the outcome is similar, mechanism is different between S. aureus and S. epidermis. For S. aureus Western Blot and qPCR analyses showed that the increase of TJ functionality is not due to a raise of TJ mRNA and protein levels, but increased levels of phospho- Occludin which result in an increase of TJ proteins at the cell cell borders, while this was not the case for S. epidermidis. Experimental data hint for a role of Toll-like receptors and cytokines for these differences.

At later time points of incubation with the bacteria, TER decreases and paracellular flux increases, arguing for the break of the TJ barrier at later time points of infection. This was not accompanied by increased cell death, but by decrease of TJ protein levels. However, again there was a difference for S. aureus and S. epidermidis concerning the influence on distinct TJ proteins.

A pretreatment of phk with an Atopic Dermatitis (AD) mimicking mix followed by infection also results in a short-term increase in TER for both bacteria, but a subsequent accelerted decrease was observed after S. aureus infection.

In conclusion, we show for the first time, that infection of primary keratinocytes not only with the commensal S. epidermidis, but also with the pathogen S. aureus results in a transient upregulation of TJ functionality, hinting for a prevention mechanism of keratinocytes against invasion of pathogens. In AD related conditions, this positive effect is reduced for S. aureus but not *S. epidermidis.*

P178

Investigating the return of *Microsporum audouinii* in Munich

A. Zink[1,2], M. Reiger[2], A. Todorova[2], H. Seidl[1], T. Biedermann[1], M. Köberle[1] and C. Traidl-Hoffmann[2] *[1]Department of Dermatology and Allergy, Technische Universität München, Munich; [2]Institute of environmental medicine, Technische Universität München, Munich*

Microsporum (M.) audouinii is a highly contagious anthropophilic fungus commonly causing tinea capitis in Germany at the beginning of the last century until the 1960s. With the introduction of the antifungal drug griseofulvin, M. audouinii became very rare in Germany. When 6 children presented with tinea capitis caused by M. audouinii within only a few weeks, we investigated an outbreak in Munich kindergartens. We analyzed social data of the patients, evaluated potential commonalities of the patients and took swabs of patients and objects in involved kindergartens for mycological analysis. Affected patients were treated with systemic and local antifungal drugs and contact person were advised to use antifungal shampoo as a prophylactic measurement. In addition, the City Health Department of Munich introduced a temporary kindergarten ban for children with M. audouinii to prevent further spreading. In summary, we found 16 children and 4 adults infected by M. audouinii, who we then successfully treated. Meticulous analysis further found out, that patient zero brought the fungus to Munich from a family vacation in Africa before it spread to fellow kindergarten children and their families. 40 weeks after the initial presentation of patient zero in our hospital, the outbreak was declared ceased causing considerable financial damage and individual challenges. Within the next years, highly infectious fungi including M. audouinii will be seen at growing numbers in Germany due to increasing exotic travel destinations as well as migration. Dermatologists, pediatricians and public health officials therefore are required to develop sufficient and sustainable solution strategies for the management and prevention of future outbreaks of highly infectious fungi.

P179

Immunization with a newly identified *Leishmania* (L.) major-specific protein (80 kDa) promotes protection in mice against infection with L. major parasites

A. I. Schermann[1], B. Lorenz[1], S. Tenzer[2] and E. von Stebut[1] *[1]University Medical Center, Johannes Gutenberg University, Mainz, Department of Dermatology, 55131 Mainz, Germany; [2]University Medical Center, Johannes Gutenberg University, Mainz, Institute of Immunology, 55131 Mainz, Germany*

Infections with sandfly-transmitted Leishmania (L.) major parasites are responsible for the manifestation of worldwide occurring human cutaneous leishmaniasis (CL). Healing of CL in immune-competent hosts is mediated by both antigen-specific CD4+ Th1 and CD8+ Tc1 cells by releasing interferon (IFN)-gamma. Due to the fact that immune-competent hosts are resistant against re-infection with the same Leishmania subspecies the development of an effective vaccine should be feasible. Despite this fact, no effective vaccine exists so far. Therefore, we aimed to identify and characterize new immunogenic, parasite-specific proteins serving as potential vaccine candidates.

Thus, we separated highly immunogenic soluble Leishmania antigen (SLA; lysate of L. major parasites) systematically into its components by biochemical and chromatographic methods for *in vitro* analysis. Some of the eluted fractions induced a strong Th1/Tc1-specific IFN-gammahigh/interleukine (IL)-4low/IL-10low cytokine profile *in vitro* upon restimulation of primed C57BL/6 lymph node cells. Next, we identified 36 l. major-specific proteins overall in reactive fractions by quantitative mass spectrometry. Four proteins were selected and recombinantly expressed in Escherichia coli. For our *in vivo* studies, both C57BL/6 mice and susceptible BALB/ c mice were immunized intradermally into one ear with 1 μg of recombinant protein combined with CpG as adjuvant followed by infection with 1000 live metacyclic L. major promastigotes in the alternate ear. Interestingly, only one protein (80 kDa) significantly promoted protection in both C57BL/6 and susceptible BALB/c mice postinfection (p.i.), whereas one additional protein (~50 kDa) also protected BALB/c mice from progressive disease. Protection against infection revealed significantly smaller ear lesion development compared to control mice, which was accompanied by significantly lower parasite loads in the spleen of C57BL/6 mice at week 6 p.i. in contrast to infected mice treated with CpG alone. Depletion of distinct T cell subsets during the immunization period of C57BL/6 mice with this newly identified 80 kDa L. major-specific protein disclosed CD4+ T cells as primarily responsible T cell subtype for healing. When intraperitoneally injected with anti-CD4 antibodies to deplete CD4+ T cells, the immunized group of mice developed ear lesions comparable to control mice after L. major infection at the time when the T cell compartment was refilled to ≥60%. In contrast, infected C57BL/6 mice with depleted CD8+ T cells during immunization showed almost no lesion progression. This result agrees with the exogenic origin of this protein and its processing and presentation via major histocompatibility complex (MHC) class II molecules to CD4+ T cells.

As this newly identified 80 kDa protein exists in both life forms of L. major parasites – promastigotes and obligate intracellular amastigotes – it represents a promising new vaccine candidate against CL and a source for protective T cell epitopes. Further analyses will focus on the underlying protective mechanism.

P180

Role of antigen dosage and adjuvant in next generation RG1-VLP broad-spectrum HPV vaccination

C. Schellenbacher[1], S. Shafti-Keramat[1], B. Huber[1], M. Skoll[1], R. Roden[2] and R. Kirnbauer[1] *[1]Medical University Vienna, Dermatology, Division of Immunology, Allergy and Infectious Diseases, 1090 Vienna, Austria; [2]The Johns Hopkins University, Pathology, Baltimore, Maryland, USA*

Licensed human papillomavirus (HPV) (2-, 4- or 9-valent) vaccines, composed of major capsid protein L1 virus-like particles (VLP), induce high-titer neutralizing antibodies, and provide type-restricted protection against persistent infection and disease with the included HPV types. The N-terminus of the minor capsid protein L2 contains highly conserved type-common motifs that may provide broad-spectrum protection against the majority of >15 different oncogenic genital types.

We have previously generated HPV16L1-VLP that present the 20 amino-acid cross-neutralization L2 epitope RG1 (RG1-VLP) repetitively on the capsid surface. Immunization has provided broad cross-protection against infection with 18 mucosal high-risk and additional low-risk HPV using aluminum hydroxide (alum) plus TLR4- agonist monophosphoryl lipid A (MPL) as adjuvant.

Complementing current GMP production the role of antigen dose and adjuvant was determined to inform planned early phase human vaccine trials.

A dose-escalation and comparison of two vs. three times vaccinations protocols were performed in NZW rabbits and mice to analyze antibody titers dependent on dose and number of immunizations. In addition, animals were immunized with RG1-VLP either without adjuvant, plus alum, or plus alum and MPL.

NZW rabbits (groups of $n = 3$) were immunized with 1, 5, 25, or 125 mcg RG1-VLP at 0, 2 and 4 months (and 0, 4 months for 5 mcg) and compared to dose of Cervarix using equivalent amounts of alum (125 mcg)/MPL (12.5 mcg) as adjuvant. Balb/c mice (groups of $n = 10$) were vaccinated with alum/MPL, 5 mcg RG1-VLP+5 mcg 18L1-VLP plus alum/MPL, dose of Cervarix, or dose of Gardasil twice (week 0, 3) or once only. Antibody responses were analyzed in 16L1-VLP ELISA or pseudovirion based neutralization assays (PBNA). Additionally, mice were challenged intravaginally with oncogenic HPV58 pseudovirions. Three times vaccination of rabbits with 5 mcg RG1-VLP induced similar antibody titers against HPV16 as the equivalent amount of Cervarix (mean 1:62.500) plus robust titers against RG1 epitope in ELISA. Two times immunizations with RG1-VLP, Cervarix(TM) or Gardasil (TM) in mice resulted in similar neutralizing antibody levels against HPV16 (1:1000–1:10.000) in PBNA, but cross-protection against vaginal challenge with unrelated type HPV58 was obtained by vaccination with RG1-VLP+18L1-VLP only.

Comparable immunogenicity between RG1-VLP and licensed HPV vaccines to the vaccine types is demonstrated, when applicated in analogous conditions. Importantly, RG1-VLP vaccination shows *in vivo* cross-protection against unrelated high-risk HPV58 and using a two dose vaccination regimen.

P181

Glucocorticoids induced cathelicidin, but fail to promote phagolysosome maturation and antimicrobial activity in human macrophages

J. Steiger[1], A. Stephan[1], M. S. Inkeles[2], S. Realegeno[2], M. Pellegrini[2], B. Brachvogel[1], R. L. Modlin[2] and M. Fabri[1] *[1]University of Cologne, Dermatology, Cologne, Germany; [2]University of California Los Angeles, Los Angeles*

Systemic glucocorticoids are extensively used in dermatology to treat inflammatory diseases. However, their chronic intake increases the risk for infections as exemplified in mycobacterial disease. Nevertheless, the effect of glucocorticoids on innate host defense against intracellular pathogens is unclear. Here, we compared the direct effects of glucocorticoids and IFN-γ on anti-mycobacterial host defense in primary human macrophages. Both triggered the expression of cathelicidin, an anti-microbial peptide critical for anti-mycobacterial response, yet through different mechanisms: in contrast to the vitamin D-dependent induction of cathelicidin by IFN-γ glucocorticoids induced cathelicidin expression independently of intracellular vitamin D metabolism. Nevertheless, only IFN-γ promoted macrophage antimycobacterial activity. Gene expression profiles of glucocorticoid and IFN-γ-treated human macrophages were investigated by weighted gene coexpression network analysis (WGCNA), identifying a module of highly connected genes that was strongly associated with IFN-γ and inversely correlated with glucocorticoid treatment. This module was linked to the biological functions 'autophagy', 'phagosome maturation' and 'lytic vacuole/lysosome', and contained members

of the vacuolar H+-ATPase (v-ATPase). We next found that glucocorticoids failed, yet IFN-γ was able to trigger expression and phagolysosome recruitment of v-ATPase subunits, as well as to promote lysosome acidification, which was dependent on autophagy induction. Taken together, we provide evidence that the induction of cathelicidin is necessary, but not sufficient for macrophage antimicrobial activity. Instead, the ability of IFN-γ to induce autophagy, as well as phagosome maturation and lysosome acidification, which are not induced by glucocorticoids, is crucial for host defense.

P182

In *Leishmania major* infections, CD8+ and CD103+ dendritic cells (DC) are important to resolve lesions

K. Kautz-Neu, B. Lorenz, A. Mauer and E. von Stebut *University Medical Centre, Johannes-Gutenberg University, Department of Dermatology, 55131 Mainz, Germany*

Cutaneous leishmaniasis is a parasitic disease transmitted by the bite of a sand fly that causes skin sores characterized by ulcerating, sometimes painful nodules of the skin. In humans and mice, the infection is resolved by a Th1/Tc1 response, which correlates with disappearance of the lesions. Persistence of small numbers of parasites in the skin and lymphoid tissues is crucial for resistance to re-infection. Defence mechanisms against L. major are exerted by different skin-derived DC subsets, including dermal DC (dDC) and epidermal Langerhans cells (LC). In experimental cutaneous leishmaniasis, LC are negative regulators of the anti- Leishmania response as they promote the expansion of CD4+Foxp3+ regulatory T cells (Treg) at the site of infection. CD103 can be used to define a population of DC found in many lymphoid organs and tissues such as intestine, lung and skin. The dermal compartment contains CD103- Langerin- and CD103+ Langerin+ DC, whereas epidermal LC are CD103neg. Batf3 (Jun dimerization protein p21SNFT) is a transcription factor required for the development of CD103+ dDC and CD8+ conventional lymph node (c)DC. Therefore, SVEV 129 Batf3$^{-/-}$ mice lack CD8+ and CD103+ DC, but have normal repertoires of other DC subsets. In this study we analysed SVEV 129 Batf3$^{-/-}$ and control mice in physiologically relevant L. major low dose infections by inoculating only 1000 infectious-stage promastigotes into the dermis. Compared to controls, lesion development was significantly enhanced in SVEV 129 Batf3$^{-/-}$ mice. In line, parasite loads were larger in L. major-infected ears and spleens of knock-out mice. Additionally, we compared immigration of inflammatory cells in infected ears. Absolute numbers and frequencies of MHCII + CD11c+ (DC), CD11b+ (myeloid lineage) and CD11b+ Ly6G+ Ly6C+ cells (neutrophils) were significantly enhanced at the site of infection in SVEV 129 Batf3$^{-/-}$. Importantly, the frequencies of CD3+ CD8+ T cells in skin draining lymph nodes (sdLN) of knock out mice were reduced compared to controls, indicating that cross presentation was impaired. IL-10 production after antigen specific restimulation was significantly enhanced in ko mice, supported by intracellular FACS staining in sdLN.

Taken together we conclude that CD103+ dDC are involved in L. major antigen transportation to the skin draining lymph node and possibly cross presentation to CD8+ T cells. In addition, CD8+ cDC may be important for cross presentation in the lymph node. Further studies using bone marrow chimeric mice will show if the cooperation of both DC subtypes is crucial to resolve lesions and prevent the development of chronic disease.

P183

High rate of spontaneous remission of intraepithelial, anal dysplasia in HIV patients under antiretroviral therapy – follow up of the IZAR cohort

A. Todorova[1,2], D. Turek[1,2], C. Spinner[1,5], G. Weirich[4], K. Kaliebe[1,3], A. Zink[1,3], C. Schwerdtfeger[1,5] and C. Traidl-Hoffmann[1,2] *[1]Interdisciplinary HIV center at University Hospital rechts der Isar (IZAR), Munich, Germany, 81675 Munich, Germany; [2]Institut for Environmental Medicine UNIKA-T, Technische Universität München, 81675 Munich, Germany; [3]Department of Dermatology and Allergy, University Hospital rechts der Isar, 80802 Munich, Germany; [4]Institute for Pathology, Technische Universität München, 81675 Munich, Germany; [5]II. Medical Department of Internal Medicine, University Hospital rechts der Isar, 81675 Munich, Germany*

Background: Changing spectrum of internal and cutaneous diseases in HIV patients with respect to cardiovascular and neoplastic diseases occurs parallel to the emerging advances of the HIV antiretroviral therapy and the prolonged expectancy of life with HIV. Chronic persistent viral infections with high risk HPV are involved in the causality and pathogenesis of squamous epithelial carcinomas. Based on the limited study data, screening- and therapy concepts for anal epithelial dysplasia in HIV patients are still controversial.

Methods: 101 HIV patients (84 men, 17 women) in regularly medical care at the interdisciplinary HIV centrum at the University Hospital rechts der Isar (IZAR) were screened at their routine visits for anal epithelial dysplasia. After a perianal inspection cell material from the anal mucosa was taken using cytobrush technique. The results were evaluated according to the Munich classification for genital epithelial dysplasia. In patients with moderate to high grade dysplasia a follow up cytology examination was performed three to six months later. For patients with dysplasia (grade IIID or IVa) proctological consultation with anoscopy was recommended. During the proctological examination biopsies for histopathology were taken from conspicuous lesions.

Results: In this study 35% of the patients ($n = 35$) showed unremarkable results. In 24% ($n = 24$) there was a discrete to moderate dysplasia (grade IIID) and in 11% ($n = 11$) a high grade dysplasia (Grad IVa).

At follow up three months later 25% ($n = 6/24$) from the patients with moderate dysplasia (group IIID) and 27% ($n = 3/11$) of the patients with high grade dysplasia (group IVa) showed unremarkable results with no cytological signs of dysplasia; in one patient there was a partial recovery from group IVa to IIID along with the recovery of his CD4 cell count. 45% ($n = 11/21$) of the patients with grade IIID dysplasia and 91% ($n = 10/11$) of the patients with IVa dysplasia were further evaluated by anoscopic examination. This evaluation showed in 81% ($n = 9/11$) of the patients in group IIID and in 80% ($n = 8/10$) of the patients in group IVa unremarkable macroscopic findings. Biopsies were performed in 18% ($n = 2/11$) of the patients of the group IIID, which showed normal squamous epithelium without signs of dysplasia. 20% ($n = 2/10$) of the patients in group IVa showed histopathologically fibropapilloma and acanthoma with Bowenoid atypias.

Conclusions: In our patient cohort we investigated cytopathologically 101 HIV patients for anal dysplasia. In one third of the patients was detected moderate to severe anal epithelial dysplasia. In single cases the results from the cytopathology were confirmed in the anoscopic examinations. At follow up one third of the patients with abnormal cytopathology showed unremarkable findings with spontaneous regression of the dysplasia. Further prospective studies in large cohorts need to be performed to evaluate the significance of the cytology brush technique in screening programs for early detection of anal carcinoma in HIV patients. Risk factors and the role of the immune reconstitution coinfections and high risk HPV may play a role in the development and clinical course of anal epithelial dysplasia in HIV patients.

P184

Altered maturation of *L. major*-containing phagosomes in dendritic cells compared to macrophages

T. Twelkmeyer[1], V. Schmidt[1], S. Tenzer[2] and E. von Stebut[1] *[1]University Medical Center Johannes Gutenberg University, Department of Dermatology, 55122 Mainz, Germany; [2]University Medical Center Johannes Gutenberg University, Institute of Immunology, 55131 Mainz, Germany*

Cutaneous Leishmaniasis is caused by protozoan of the genus Leishmania that parasitize in phagocytic cells. Upon inoculation in the upper dermis as flagellated infectious stage promastigotes by an infected sand fly, it encounters skin-resident macrophages (MΦ). After CR3-mediated internalization, *L. major* efficiently transforms into the obligate intracellular amastigotes and replicate without inducing inflammation ('silent phase'). Released from ruptured MΦ, amastigotes subsequently infect dendritic cells (DC). FcγRI/III-mediated uptake leads to DC activation, parasite antigen processing, migration to draining lymph nodes and priming and activation of T cells. Finally, IFNγ-producing antigen-specific T-cells induce parasite clearance by activating infected MΦ to produce intracellular NO. Thus, DC and MΦ get in contact with parasites early in the disease, but their behavior towards infection and the intracellular fate of Leishmania differs dramatically, which may be caused by intracellular events involved in parasite internalization and the molecular composition of parasitophorous vacuoles (PV). To better understand phago(lyso)somal function in *L. major*-containing PVs in DC compared to MΦ, we first analyzed parasite replication in DC and MΦ after infection with infectious stage promastigotes and obligate intracellular amastigotes. CFSE-labelled parasites isolated from disrupted cells were examined by flow cytometry. After internalization by MΦ, promastigotes exhibited a shift in size and granularity indicating intracellular transformation into amastigotes within the cells. Furthermore, a decrease in amastigote CFSE staining indicated a faster replication of parasites in DC compared to MΦ within 22 h. Next, flow cytometry was used to assess changes in the expression levels of endocytic molecules in infected compared to uninfected cells. For MΦ infected with amastigotes, we detected a slow decrease in the expression of the early and late endosomal tracers EEA1 and Rab7 over time, whereas the lysosomal marker Lamp2 remained unaltered for 18 h post infection. In contrast, in infected DC, the expression levels of all maturation markers increased over time after incubation with amastigotes. In addition, expression of characteristic phagosomal maturation markers restricted to amastigote containing PVs was determined by immunofluorescence labeling (IF) and confocal microscopy over a time of 6 h post infection. By IF, enrichment for all tested endosomal markers on parasitecontaining PVs in MΦ was observed, whereas in DCs decreased expression of early and late markers and increased expression of Lamp2 was found. In summary, these findings suggest that the phagosomal maturation of PVs harboring amastigotes in DC proceeds slower than in MΦ and that the upregulation of endosomal markers is restricted to parasite-containing PVs. Further experiments with latex beads will have to assess the specificity of this effect to engulfment of life parasites. These analyses will contribute to our understanding of the molecular mechanisms behind their different behavior of phagocytes in infection with Leishmania.

P185 (O05/06)

Beta-defensin 14 deficieny in mice leads delay in skin permeability barrier repair, but doesn't influence infection rate with *Staphylococcus aureus*

E. Proksch[1], C. Neumann[1], C. Walker[2], W. Müller[2], J. Harder[1] and N. Graumann[1] *[1]University of Kiel, Department of Dermatology, 24105 Kiel, Germany; [2]University of Manchester, Faculty of Life Sciences, M13 9PL Manchester, UK*

Mouse beta-defensin 14 (mBD-14), the mouse orthologue of human beta-defensin 3, has a broad antimicrobial activity and exhibits immunomodulatory activities such as chemotactic activity for T-cells. Inflammatory signals including cytokines and epidermal proliferation are important for skin barrier repair. We asked (a) whether a deficiency in mBD-14 expression leads to a delay in permeability barrier repair and (b) whether this deficiency does influence the rate of bacterial infection. Permeability barrier disruption in mBD-14 deficient mice was induced by tape stripping and repair of the skin barrier was monitored as recovery in TEWL. Recombinant mBD-14 or the vehicle was applied topical on back skin of mBD-14 deficient mice after skin barrier disruption. In a second set of experiments shaved mBD-14 deficient mouse skin was infected with Staphylococcus aureus ALC2906 (10^2 CFU/15 μl PBS). covered with Finn Chambers. The infected skin was treated with several concentrations of mBD14 in PBS with 0.01% acetic acid or the vehicle. After 24 h skin biopsies were obtained and analyzed by histology and immuno-histology, also bacterial counts were performed.

We found that mBD-14-deficient mice exhibited a delay in barrier repair at 1–24 h after tape-stripping as compared to wild-type mice. Topical application of a solution of 1% recombinant mBD-14 partially reversed the delay in permeability barrier repair. Barrier disruption resulted in an inflammatory cell infiltrate and IL-1 expression in wild type mice, but much less in mBD-14 deficient mice. Barrier disruption by shaving the skin was sufficient to induce infection with Staphylococcus aureus in mBD-14 deficient mice. The infection rate, bacterial count and neutrophil infiltrate as a marker of bacterial infection was unchanged in mBD-14 deficient mice compared to control mice. Also, application of various concentrations on recombinant mBD-14 didn't influence the infection rate neither in mBD-14 deficient mice nor in control mice. We suggest that delay in permeability barrier repair in mBD-14 deficient mice may be related to the known chemoattractant and proinflammatory activity of this defensin. Surprisingly, mBD-14 didn't influence bacterial infection rate. It is known that mice get seldom infected with Staphylococcus aureus and may have a redundant system to fight this bacterial species.

P186

The therapeutic potential of *Pelargonium sidoides* extract EPs 7630 in the antimicrobial skin defense

K. Witte[1,2], E. Koch[3], E. Witte[1,4], H. Volk[2,5], K. Wolk[1,4] and R. Sabat[1,4] *[1]University Hospital Charité, Interdisciplinary Group of Molecular Immunopathology, Dermatology/Medical Immunology, 10117 Berlin, Germany; [2]University Hospital Charité, Berlin-Brandenburg Center for Regenerative Therapies, 13353 Berlin, Germany; [3]Dr. Willmar Schwabe GmbH & Co. KG, Preclinical Research, 76227 Karlsruhe, Germany; [4]University Hospital Charité, Psoriasis Research and Treatment Center, 10117 Berlin, Germany; [5]University Hospital Charité, Institute of Medical Immunology, 13353 Berlin, Germany*

Viral and bacterial skin infections are a severe complication in several dermatological diseases like atopic dermatitis, cutaneous T-cell lymphoma and in the context of skin wound healing. Moreover, especially in aging patients cutaneous antiviral and antibacterial defense mechanisms are comprised. Today, therapies most commonly include virostatics and antibiotics, whereby their effectivity might be limited in case of development of resistances.

Hence, as therapy options are still limited, novel anti-infective treatment strategies are needed in dermatology, which focus on a gentle enhancement of the cutaneous immunity.

Pelargonium sidoides is a medical herb used very frequently for the treatment of respiratory tract infections. The anti-infective properties of respective herbal extracts (EPs 7630) are described to be mainly mediated by inhibition of viral attachment and spreading as well as of bacterial adherence. However, it is still unknown whether EPs 7630 might influence the skin immunity and if this extract is able to exert direct cellular effects.

To investigate the role of EPs 7630 in the cutaneous antimicrobial defense we first aimed to test its ability to target the immune system of the skin. Indeed, we identified, that EPs 7630 directly targets monocytes, and predominantly induced the production of IL-6 and TNF-alpha in these cells. Moreover, we observed that EPs 7630 treatment leads to the induction of IL-22 and IL-17, which was attributed to non- T cells. Consequently, IL-22 application of mice strongly increased the keratinocyte production of antibacterial proteins (ABPs).

Regarding its mode of action, we could show that EPs 7630 provoked the activation of MAP kinases and inhibition of p38 strongly reduced the monocyte TNF-alpha production induced by EPs 7630. Furthermore, the pretreatment of blood immune cells with EPs 7630 lowered their secretion of TNF-alpha and caused an IL-6 dominant response during second stimulation with viral or bacterial infectionmimicking agents. The latter results, together with the ability of IL-22, IL-17, TNFalpha and IL-6 to induce ABPs, might implicate a role of EPs 7630 in promoting neutrophilic granulocyte generation, skin remodeling and antimicrobial skin protection.

In summary, our results suggests, that cutaneous application of EPs 7630 might have therapeutic potential in the treatment of skin infections.

Pharmacology

P187

Skin rashes induced by vemurafenib are caused by a non-allergic mechanism

H. C. Friedrich[1], P. A. Gerber[1], A. Kislat[1], K. Schindler[2,3], N. Klossowski[1], S. A. Braun[1], P. Chapman[2], M. E. Lacouture[2], B. Homey[1] and S. Meller[1] *[1]Heinrich-Heine-University, Department of Dermatology, Düsseldorf, Germany; [2]Memorial Sloan-Kettering Cancer Center, Department of Medicine, New York, USA; [3]Medical University of Vienna, Department of Dermatology, Vienna, Austria*

Introduction: The BRAF protein kinase inhibitor vemurafenib was recently approved for the treatment of advanced malignant melanoma. Although it is well tolerated, cutaneous adverse effects, including inflammatory rashes or secondary skin tumors, have been reported in about seventy percent of patients under vemurafenib therapy and occasionally result in discontinuance of treatment.

Materials and Methods: To characterize vemurafenib-induced rashes we performed immunohistochemical and gene expression analysis of lesional skin sections of vemurafenib-treated patients. Lymphocyte activation tests (LAT) were conducted to detect vemurafenib-specific T cells. Furthermore, we stimulated T cells from healthy donors with different concentrations of vemurafenib and evaluated the gene expression profile on mRNA and protein levels. Finally, rechallenges with vemurafenib in patients were performed to establish a protocol for the management of respective rashes.

Results: Vemurafenib-induced skin rashes are characterized by a massive infiltration and clustering of T cells (CD4+ and CD8+), CD68+ macrophages, mast cells as well as intraepidermal CD1a+ Langerhans cells, whereas eosinophils were not detected. Additionally, we detected a strong upregulation of the proinflammatory cytokines TNF-alpha, IFN-gamma and IL-1beta and several chemokines including CCL2, CCL5, CCL27 and CXCL14 in comparison to healthy skin. *In vitro*, T cells respond with high IFN-gamma and IL-17A expression after both 6 h and 24 h. Nevertheless, vemurafenib-specific T cells were not found in patients using LAT. Regarding the protocol of rechallenge; the response rate after reintroduction of vemurafenib without adverse effects was ninety percent.

Conclusions: The composition of the inflammatory infiltrate, the chemokine/cytokine expression pattern, and the lack of vemurafenib-specific T cells indicate that vemurafenib-associated rashes are caused by a pharmacologic non-allergic mechanism, rather than an allergic hypersensitivity against the drug. Clinical proofof- concept analyses demonstrate that affected patients may benefit from the rechallenge regimen.

P188

Ex vivo microdialysis used for the preclinical assessment of anti-inflammatory therapy

N. Doege[1,2], S. Hoenzke[2], F. Schumacher[3], B. Balzus[2], M. Colombo[2], S. Hadam[1], F. Rancan[1], U. Blume-Peytavi[1], A. Schindler[2], E. Ruehl[2], P. Skov[4], M. Church[5], S. Hedtrich[2], B. Kleuser[3], R. Bodmeier[2] and A. Vogt[1] *[1]Department of Dermatology, Venerology and Allergology, Charité – Universitätsmedizin Berlin, Germany; [2]Department of Biology, Chemistry and Pharmacy, Freie Universität Berlin, Germany; [3]Department of Nutritional Toxicology, University of Potsdam, Germany; [4]RefLab, Copenhagen, Denmark; [5]Department of Dermatology and Allergy, Charité-Universitätsmedizin Berlin, Germany*

Cutaneous inflammation and disturbed skin barrier are found in a large number of chronic skin diseases. Patients would greatly benefit from novel delivery systems, which target such inflammatory cell infiltrates. We established a standardized physical and chemical barrier disruption model based on *ex vivo* human skin to assess preclinically whether we can deliver the model anti-inflammatory drug dexamethasone (DXM) loaded on nanocarrier systems to a specific skin layer. We applied *ex vivo* microdialysis to compare drug penetration in parallel with cytokine production on intact versus barrier-disrupted skin at 6, 12, and 24 h after topical administration. DXM was quantified in the microdialysis eluates and whole tissue samples using a highly sensitive and specific liquid-chromatography – tandemmass spectrometry (LC-MS/MS) approach. Extracted cytokines collected from skin surface and whole tissue samples were analyzed using ELISA as well as screening assays. Comparison of 0.05% DXM in cream to DXM applied as nanocrystals or ethylcellulose carriers, revealed marked differences in the penetration of DXM across chemically versus physically damaged skin. Furthermore, less DXM was extracted from the dermis when it was applied on nanocarriers, which suggests a longer exposure of diseased epidermis to the drug. Reduced dermal concentration indicates the potential of these nanocarriers to reduced corticosteroid side effects in the dermis. Penetration of DXM released from nanocrystals occurred faster than the penetration of DXM released from ethylcellulose carriers. First evidence for effective regulation of cytokines in this short-term *ex vivo* skin model were obtained.

In summary, *ex vivo* human skin incubated under standardized conditions for up to 48 h combined with microdialysis as well as specific and sensitive analytical methods (LC-MS/MS, ELISA, screening assays) is a promising model for preclinical assessment of penetration and efficacy of novel drug delivery systems, because it yields drug release as well as cytokine level and safes resources by maximizing the read-out obtained from each donor skin sample.

Photobiology

P189

Protective efficacy of a licochalcone A containing sunscreen in the high energy visible spectral range *in vivo*

M. E. Darvin[1], S. Schanzer[1], J. Lademann[1], M. Tesch[2], A. Bueger[2], F. Rippke[2] and M. C. Meinke[1] *[1]Charité – Universitätsmedizin Berlin, Center of Experimental and Applied Cutaneous Physiology, Department of Dermatology, Venerology and Allergology, Berlin, Germany; [2]Beiersdorf AG, Research and Development, Hamburg, Germany*

Various experimental investigations have shown that free radicals including reactive oxygen species are not only produced in the UV but also in the visible (VIS) spectral range [1]. Recent *in vivo* studies confirm the generation of free radicals in the skin subsequent to VIS exposure, which was measured by the degradation of carotenoid antioxidants in human skin post irradiation [2]. UV filters designed for modern sunscreens efficiently protect skin from UVA and UVB radiation but their action spectrum does not cover longer wavelengths. Therefore, an additional skin protection in the VIS ranges is also required namely in people with darker skin complexion or photodermatoses [3,4]. Antioxidants are a main option to protect the skin in the VIS range [5]. The compound licochalcone A (Lic A) is known for its antiinflammatory and antioxidant effects [6]. In this study, the protective efficacy of a sunscreen formulation containing Lic A was investigated *in vivo* in the visible range (400–700 nm, with a maximum at 440 nm) using resonance Raman spectroscopy (RRS). It was compared to an identical formulation without Lic A in a double blind pilot study performed on six healthy volunteers aged between 20 and 60 years. The sunscreens were topically applied on the volunteers' forearms, and after 1 h of absorption the initial carotenoid values were measured. After irradiation with 100 J/cm^2 the measurements were repeated. At the unprotected area and the area treated with the sunscreen without Lic A the carotenoid content dropped significantly by 15%, whereas the carotenoids in the area treated with the Lic A containing sunscreen on average did not change. This illustrates that a protection in the visible spectral range is achievable by sunscreens *in vivo* when a potent antioxidant compound like Lic A is applied.

References: [1] Zastrow L, Groth N, Klein F, Kockott D, Lademann J, Renneberg R, Ferrero L: The missing link–light-induced (280–1600 nm) free radical formation in human skin. Skin Pharmacol Physiol 2009;22:31–44.

[2] Vandersee S, Beyer M, Lademann J, Darvin ME: Blue-violet light irradiation dose dependently decreases carotenoids in human skin, which indicates the generation of free radicals. Oxidat. Med. Cell. Longev. 2015: 579675.

[3] Duteil L., Cardot-Leccia N., Queille-Roussel C., Maubert Y., Harmelin Y., Boukari F., Ambrosetti D., Lacour J.-P., Passeron T.: Differences in visible lightinduced pigmentation according to wavelengths: a clinical and histological study in comparison with UVB exposure. Pigment Cell Melanoma Res. 2014, 27: 822–826.

[4] Montaudie H., Lacour J.-P., Rostain G., Duteil L., Passeron T.: Solar urticaria to visible light triggered by light-emitting diode therapy. J. Am. Acad. Dermatol. 2014, 71 (3): e74–e75.

[5] Kolbe L.: How much sun protection is needed? Are we on the way to full-spectrum protection? J. Invest. Dermatol. 2012, 132: 1756–1757.

[6] Kuehnl J., Roggenkamp D., Gehrke S.A., Staeb F, Wenck H., Kolbe L., Neufang G.: Licochalcone A activates Nrf2 *in vitro* and contributes to licorice extract-induced lowered cutaneous oxidative stress *in vivo*. Exp. Dermatol. 2015, 24: 42–47.

P190

Impact of photodynamic therapy on T cellular immune responses in oral lichen planus

R. Stein[1], R. Cosgarea[2], J. Sharif[2], N. Arweiler[2], M. Hertl[1] and T. Schmidt[1] *[1]Philipps-University Marburg, Department of Dermatology and Allergology, Marburg, Germany; [2]Philipps-University Marburg, Department of Periodontology, Marburg, Germany*

Oral lichen planus (OLP) is a common T-cell mediated autoimmune disease which affects the oral mucous membranes. The inflammatory infiltrate in mucosal lesions is dominated by CD8+ and CD4+ T lymphocytes which presumably contribute to degeneration of basal keratinocytes which is critical for disease development. Currently, OLP is treated with non-specific topical or systemic glucorticoids or immune modulators such as tacrolimus. We here studied the anti-inflammatory effect of photodynamic therapy (PDT) as a non-invasive, easy-to-use and safe alternative therapy option in OLP. Although PDT treatment has been shown to exert anti-inflammatory effects, its impact on adaptive immune responses has not been thoroughly studied. Nine OLP patients received four consecutive PDT treatments of the buccal mucosa within 2 weeks and their peripheral T cell subsets, plasma, and saliva were analysed pre- and post treatment. *Ex vivo*, the numbers of interferon-γ (IFNγ), interleukin-5 (IL-5) and interleukin-17a (IL-17a)-secreting T cells were determined by ELISpot, cytokine and chemokine levels of plasma and saliva were determined by ELISA. There was a significant increase of peripheral γ/δ T cells ($P = 0.0288$) and decrease of IL-17a-secreting T cells ($P = 0.0245$) which was associated with a strong decrease of plasma levels of the chemoattractant, CXCL10 ($P = 0.0021$). Moreover, there was a decrease of peripheral activated CD8+ T cells (CD3+CD137+) upon PDT treatment ($P = 0.0262$). These findings strongly suggest that local PDT treatment of the oral mucosa exerts profound anti-inflammatory effects which lead to a reduction of pro-inflammatory CD8+ T cells. Moreover, the increase of peripheral γ/δ T cells may be linked to a decrease of CXCL10 which drives extravasation of pro-inflammatory cells in OLP.

P191

The aryl hydrocarbon receptor (AHR) critically contributes to UVB-induced skin carcinogenesis

M. Pollet[1], S. Shaik[1], K. Frauenstein[1], J. Tigges[1], T. Douki[2], C. Esser[1], J. Krutmann[1] and T. Haarmann-Stemmann[1] *[1]IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany; [2]CEA-Grenoble, Grenoble, France*

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor known to mediate the toxic effects of dioxins, PAHs and related environmental pollutants. In its inactive state, AHR is part of a cytosolic multiprotein complex. Upon ligand binding, this complex dissociates, AHR shuttles in the nucleus, dimerizes with ARNT and binds to dioxin-responsive elements in the promoter of target genes to enforce their transcription. In epidermal keratinocytes (KC), AHR signaling also occurs as a consequence of UVB irradiation. Specifically, UVB radiation-induced AHR activation results from the absorbance of UVB rays by cytosolic tryptophan and the subsequent generation of 6-formylindolo [3,2b]carbazole. This tryptophan photoproduct can bind to AHR and thereby activates downstream signaling processes.

We have previously shown that the AHR serves an anti-apoptotic function in human KC and murine skin. A key role in the AHR-mediated anti-apoptosis seems to be a reduction of the p27 tumor suppressor protein. Inhibition of AHR, either by chemical antagonists or RNAi, leads to an increase in p27 protein level, which is associated with a reduced proliferation and enhanced apoptosis susceptibility. As apoptosis is probably the most important mechanism in the epidermis restraining skin carcinogenesis, our current study aims to identify (i) how the AHR controls p27 protein level in KC and (ii) to which extent the AHR contributes to photocarcinogenesis in SKH-1 hairless mice.

Our results demonstrate that the AHR supresses the proteasomal degradation of p27 in KC and thereby contributes to UVB-induced skin carcinogenesis. Specifically, we found that the enhanced p27 protein level in AHR-knockdown KC (HaCaTshAHR) was associated with a reduced phosphorylation of AKT. Overexpression of myristoylated (active) AKT decreased the p27 protein level in HaCaT-shAhR, whereas exposure of AHR-proficient HaCaT-EV to phosphoinositide-3-kinase (PI3K) inhibitors reduced AKT phosphorylation and increased the p27 level. The alterations in p27 level and AKT phosphorylation in HaCaT-shAHR were associated with a reduced phosphorylation of EGFR, indicating that the AHR triggers basal EGFR activity to regulate p27 protein level. In fact, stimulation of AHR-proficient HaCaT KC with BaP or EGF led to a reduction of the p27 protein level, which was blunted in the presence of a proteasome inhibitor.

To assess the *in vivo* relevance of these findings, we next investigated the p27 protein level in the skin of SKH-1 mice. Indeed, we found an elevated amount of p27 protein in the skin of AHR-null mice, which was associated with an increased occurrence of apoptosis and an accelerated clearance of cyclobutane pyrimidine dimers (CPD) upon UVB irradiation, thus strongly indicating that the AHR contributes to photocarcinogenesis. Therefore, we performed a chronic UVB irradiation study in AHR-proficient and AHR-null SKH-1 hairless mice. In contrast to control mice, AHR-null mice developed approx. 50% less cutaneous squamous cell carcinomas, indicating that the AHR attenuates CPD clearance *in vivo* and thereby critically contributes to photocarcinogenesis.

Our findings identify the AHR as a critical regulator of the p27 tumor suppressor protein and a promising molecular target for the prevention of UVB-induced skin malignancies.

P192 (O06/02)

Cockayne syndrome (CS) is characterized by lysosomal dysfunction and a disturbance of autophagic flux: results from three different species

M. Majora, M. Schneider, I. Uthe, A. Schiavi, N. Ventura and J. Krutmann *IUF-Leibniz Institut für umweltmedizinische Forschung, 40225 Düsseldorf, Germany*

Cockayne syndrome (CS) is a rare hereditary disease mainly caused by mutations in the CSB gene. It is usually classified as a nucleotide repair deficiency syndrome because CSB fibroblasts are defective in transcription coupled DNA repair and CS patients show skin UV hypersensitivity. Also, we have previously reported that CSB-deficient SKH1 mice, upon chronic UV irradiation, develop a striking skin phenotype including massive skin inflammation, development of skin fibrosis and epidermal hypertrophy as well as a dramatic loss of subcutaneous fat. Importantly, besides skin symptoms, clinical hallmarks of CS patients include neurological abnormalities and dwarfism, indicating that the CSB protein may have biological functions beyond its role in DNA repair. In keeping with this assumption is our previous notion that treatment of CSB-deficient mice with the pan HDAC inhibitor SAHA completely rescued their skin phenotype. In the present study we analyzed the mechanism(s)

underlying this therapeutic effect. We found that development of the skin phenotype in CSB deficient mice was associated with a blockade in autophagy due to lysosomal dysfunction. Accordingly, immunohistochemical analysis showed a massive accumulation of the autophagy-related proteins LC3B, p62, as well as of ubiquitin and LAMP2 positive lysosomes and cathepsins, in particular in the degenerated subcutaneous tissue of irradiated CSB mice, indicating impaired autophagy and lysosomal dysfunction. Similar to mice, an accumulation of LC3B, p62, lysosomes and cathepsins was present in CSB-deficient primary human skin human fibroblasts. In these cells, electron microscopy showed the presence of irregularly shaped and dilated lysosomes which were partially filled with nondegraded material, i.e. lysosomes reminiscent of cells from patients with lysosomal storage diseases. In addition, in the nematode C. elegans CSB knockdown resulted in decreased formation of LC3B positive autophagosomes and an enhanced accumulation of p62 agglomerates and Nile Red dye consistent with lysosomal lipid storage due to autophagic blockade. Most importantly, oral treatment of CSBdeficient mice did not only rescue the skin phenotype, but prevented accumulation of LC3B, p62, and ubiquitinated proteins in skin suggesting that SAHA treatment worked by epigenetic enhancement of autophagic flux. Accordingly, in CSB-deficient human fibroblasts SAHA improved lysosomal function and reduced accumulation of p62 agglomerates in a lysosome-dependent manner, and in CSB knockdown C. elegans SAHA prevented p62 and Nile Red accumulation.
These results provide evidence from three different species that CSB deficiency causes lysosomal dysfunction and a subsequent disturbance of autophagy. Our observation that HDAC inhibition can overcome these deficiencies and at the same time significantly improve the clinical phenotype of CSB deficient mice indicates that (i) these disturbances are clinically relevant and (ii) that they are due to epigenetic dysfunction.

P193

Inflammasome-intrinsic caspase-5 interferes with UVB-triggered IL-1 beta activation in epidermal keratinocytes

E. Hattinger, S. Koglin, D. Bureik, T. Ruzicka and R. Wolf *Ludwig Maximilian University, Department of Dermatology and Allergology, 80337 Munich, Germany*

The skin is the first line of defense and protects against physical stress, such as environmental irradiation. UVB induces a cutaneous inflammation through IL-1 beta release with subsequent infiltration of inflammatory cells. In epidermal keratinocytes, UVB leads to activation of caspase-1 dependent inflammasomes that are required for IL-1beta activation. In this study, we showed that UVB irradiation induces caspase-5 and amplifies the IFNgamma-mediated inflammasome expression and IL-1 beta release by epidermal keratinocytes. Under this condition, caspase-5 however interfered with the regulation of IL-1beta, caspase-1 and AIM2 in keratinocytes and suppressed the IL-1beta release inflammasome-independently. Data indicate that the IL-1 beta production is dampened by inflammasome-intrinsic components and may contribute to the control of UVB-triggered Th1-inflammatory skin diseases.

P194

Caspase-5 rescues UVB-dependent IL-1beta production by epidermal keratinocytes

E. Hattinger and R. Wolf *Ludwig Maximilian University, Department of Dermatology and Allergology, 80337 Munich, Germany*

IL-1beta is a potent pro-inflammatory mediator induced in inflammatory skin diseases and activated by several environmental triggers. In epidermal keratinocytes, proinflammatory IFNgamma regulates inflammatory caspases, which are activated by UVB irradiation to cleave IL-1 beta. Apoptosis-associated speck-like protein containing a CARD (ASC) is an adaptor protein, which is required for activation of UVB-dependent IL-1beta release by caspase-1. Inflammatory caspase-5 can function independently of ASC to activate IL-1, and we hypothesized that caspase-5 rescues an UVB-induced IL-1beta production in the absence of ASC. Here, cultured keratinocytes were UVB irradiated in the presence of IFNgamma to induce IL-1beta release into the supernatant as measured by ELISA. ASC levels remained unaffected but when suppressed by siRNA interference, IL-1 beta was induced and increasingly released by keratinocytes. Under these conditions, caspase-5 expression was induced compared to caspase-1, which indicates a caspase-5 dependent IL-1beta production in the absence of ASC. Together, data suggest an ASC-independent mechanism for IL-1beta regulation in keratinocytes through caspase-5, which may serve as an important backup system in the skin to secure an IL-1 beta mediated response upon environmental stimuli, such as UVB irradiation.

P195

Does indoor tanning increase the risk for cutaneous melanoma? A meta-analysis and systematic review of the literature

B. Burgard[1], J. Schöpe[2], J. Ordonez-Mena[3], S. Pilz[4,5], R. Saternus[1], S. Reichrath[1], C. Schiekofer[1], W. März[6], S. Wagenpfeil[2], T. Vogt[1] and J. Reichrath[1] *[1]Saarland University, Department of Dermatology, 66421 Homburg, Germany; [2]Saarland University, Institute for Medical Biometry, Epidemiology and Medical Informatics, 66421 Homburg, Germany; [3]Deutsches Krebsforschungszentrum, Division of Clinical Epidemiology and Aging Research (C070), Heidelberg, Germany; [4]VU University Medical Center, Department of Epidemiology and Biostatistics, EMGO Institute for Health and Care research, Amsterdam, The Netherlands; [5]Medical University of Graz, Department of Internal Medicine, Division of Endocrinology and Metabolism, Graz, Austria; [6]Ruperto-Carola University of Heidelberg, Mannheim Institute of Public Health, Heidelberg, Germany*

There is an ongoing debate whether indoor tanning may increase the risk for primary cutaneous malignant melanoma. To address this question, we have now performed a meta-analysis and systematic review of the literature, searching two databases (Medline, Web of Science). We identified observational studies that reported odds or hazard ratios for the association of ever use of indoor tanning ($N = 32$), first use of indoor tanning at younger age ($N = 10$) and high use of indoor tanning ($N = 16$) with melanoma risk. No interventional trials were found. Heterogeneity across included studies was assessed using the I^2 statistics and was taken into account by performing a random-effects meta-analysis. Moreover, sensitivity analyses were conducted to verify the robustness of our pooled results and to explore possible causes of heterogeneity. Quality of individual studies was investigated using a modification of the Newcastle-Ottawa quality assessment scale and according to a grading system for recommendations in evidence based medicine. The overall evidence level and quality of studies identified was low, due to the severe limitations of many of the observational studies, including unobserved or unrecorded confounding.

P196

PUVA pretreatment leads to reduced induction of inflammation in the imiquimod model of psoriasis

N. Shirsath[1,2], G. Mayer[2], R. Frei[3], J. Ober[1], A. Heinemann[3] and P. Wolf[2] *[1]Center for Medical Research, Medical University of Graz, Austria, 8010 Graz, Austria; [2]Research Unit for Photodermatology, Department of Dermatology and Venereology, A-8036 Graz, Austria; [3]Department of Experimental and Clinical Pharmacology, Medical University of Graz, 8010 Graz, Austria*

Psoriasis is a chronic inflammatory skin disease where cells of innate and adaptive immune system are relevant players in initiation and progression of disease. Here, we investigate how 8-methoxypsoralen plus UVA (PUVA) pretreatment affects key mediators in the initiation and progression of psoriasis using the imiquimod (IMQ) psoriasis mouse model. Mice were pretreated with topical 0.25 J/cm^2 PUVA twice a week for 2 weeks with a gap of 2 days (group 2) and a prolonged gap of 8 days to start of IMQ treatment (group 3), while to the control mice IMQ was applied only (group 1). To check the effect of PUVA on responsiveness of neutrophiles, bone marrow cells were isolated at different time point as indicated. Skin, blood, serum, spleen and lymph nodes were collected at the end for analysis. In PUVA pretreated mice (group 2) there was significantly reduced inflammation up to a 70%, as measured by macroscopic skin swelling ($P < 0.05$) and epidermal thickness ($P < 0.001$) compared to control. This observation was linked with significant and marked reduction of spleen size ($P = 0.0002$), as well as in number of APCs like pDC (120G8 +) and B cells (B220 +) in spleen and lymph nodes. PUVA not only lowered the numbers of neutrophiles ($P = 0.04$) and monocytes ($P = 0.059$) in the blood but also reduced the responsiveness and migration of neutrophiles towards IL-8. Also, the effect of PUVA was lost when a prolonged gap of 8 days to start of IMQ treatment was applied (group 3). Taken together, this data proves that PUVA pretreatment delays and reduce induction of inflammation due to imiquimod. The fact that pretreatment of PUVA is effective in the model points to an indirect therapeutic mechanism related to affection of immune cells in particular of innate immunity.

Pruritus

P197

ET-1 induced itch and its signalling trait

J. Buddenkotte[1,2], M. Kido-Nakahara[3], C. Kempkes[4], T. Akiyama[5], T. Buhl[6], F. Nunes[7], M. Sulk[1], M. Furue[3] and M. Steinhoff[8] *[1]University Hospital Muenster, Dermatology, 48149 Muenster, Germany; [2]University Hospital Düsseldorf, Dermatology, Düsseldorf; [3]Kyushu University, Graduate School of Medical Sciences, Dermatology, Fukuoka, Japan; [4]UCSF, Dermatology, San Francisco, USA; [5]UCD, Neurosciences, Davis, USA; [6]University of Göttingen, Dermatology, Göttingen, Germany; [7]University of Muenster, Institutes of Pharmacology and Toxicology, Muenster, Germany; [8]University College Dublin, Department of Dermatology and Charles Institute for Translational Dermatology, Dublin, Ireland*

Pruritus is a common but poorly understood symptom in various skin and systemic diseases. Endothelin 1 (ET-1) evokes histamine-independent itch in mice and men through activation of its receptor endothelin A receptor (ETAR). Here, we have identified neural endothelin-converting enzyme 1 (ECE-1) as a key regulator of ET-1-induced pruritus. We show that ETAR, ET-1, and ECE-1 are expressed and colocalize in human peripheral nerves and mouse dorsal root ganglia (DRG) neurons. ET-1 induces internalization of ETAR to ECE-1-containing endosomes, where ECE-1 regulates ETAR recycling and activation of ERK1/2. In an *in vivo* itch model, ET-1 elicits scratching behavior that is modulated by ECE-1 inhibition and abrogated by ERK1/2 inhibition. Iontophoretic *in-vivo* analyses demonstrate the pruritogenic, partially histamine-independend, potency of ET-1 in humans. Immunohistochemical evaluation of skin from chronic itch patients confirms an upregulation of ET-1. Together, our data in detail dissect the signalling pathway of ET-1 in sensory neurons to induce itch in mice and implicate the ET-1/ECE-1/ERK1/2 pathway as a therapeutic target to treat yet intractable pruritus in humans.

P198

The pruritus- and TH2-associated cytokine Interleukin-31 promotes growth of sensory nerves

M. Feld[1], R. Garcia[2], S. Katayama[3], J. Buddenkotte[1], K. Lewis[2], G. Muirhead[4], P. Hevezi[5], K. Plesser[1], H. Schrumpf[4], O. Sergeeva[6], H. W. Müller[7], S. Tsoka[4], J. Kere[3], S. R. Dillon[2], M. Steinhoff[8] and B. Homey[1] *[1]University Hospital Düsseldorf, Dermatology, Düsseldorf, Germany; [2]ZymoGenetics, (a Bristol-Myers Squibb Company), Seattle, USA; [3]Karolinska Institutet, Biosciences and Nutrition, Huddinge, Sweden; [4]King's College London, Informatics, London, UK; [5]University of California, Physiology and Biophysics, Irvine, USA; [6]Heinrich-Heine-University, the Department of Neurophysiology, Düsseldorf, Germany; [7]Heinrich Heine University, the Molecular Neurobiology Laboratory, Neurology, Düsseldorf, Germany; [8]Department of Dermatology and UCD Charles Institute for Translational Dermatology, Dublin, Ireland*

Pruritus is a cardinal symptom of atopic dermatitis. Next to direct neuronal activation also an increased cutaneous sensory network is thought to contribute to pruritus. Although the immune cell – IL-31 – neuron axis has been implicated in severe pruritus during atopic skin inflammation, IL-31′s neuropoietic potential has not been evaluated yet. Here, we aimed to analyze the IL-31-related transcriptome in sensory neurons and to investigate whether IL-31 promotes sensory nerve fiber outgrowth *in vitro* and *in vivo*. During *in vitro*-analyses, primary sensory neuron culture systems were subjected to whole transcriptome sequencing, Ingenuity pathway analyses, immunofluorescence and nerve elongation as well as branching assays. *In vivo*, we investigated the cutaneous sensory neuronal network in wildtype, Il31-transgenic and IL-31-pump equipped mice.
Both, transgenic Il31-overexpression and s.c delivery of exogenous IL-31 induced a significant increase in the cutaneous nerve fiber density in lesional skin *in vivo*. Transcriptional profiling of IL-31-activated DRG neurons revealed enrichment for genes promoting nervous system development, neuronal outgrowth and negatively regulating cell death. Moreover, the growth cones of primary small diameter DRG neurons showed abundant IL-31RA expression. Indeed, IL-31 selectively promoted nerve fiber extension only in small diameter neurons. STAT3 phosphorylation mediated IL-31-induced neuronal outgrowth and pharmacological inhibition of STAT3 completely abolished this effect. In contrast, active TRPV1 channels were dispensable for IL-31-induced neuronal sprouting.
The pruritus- and TH2-associated novel cytokine IL-31 induces a distinct transcriptional program in sensory neurons leading to nerve elongation and branching *in vitro* and *in vivo*.

P199

Scratching the surface of prurigo nodularis hyde: a hypothesis on its development

G. Nikolakis, A. Lyakh, M. Brunner, I. Karagiannidis and C. C. Zouboulis *Dessau Medical Center, Departments of Dermatology, Venereology, Allergology and Immunology, 06847 Dessau, Germany*

Subacute prurigo (prurigo simplex subacute, PSS) is a disease of severely itching papular, sometimes urticarial, inflammatory lesions. The primary lesions (serous papules) are rarely observed because of of the intense itching, resulting quickly in secondary excoriations. In contrast, prurigo nodularis Hyde (PNH) is a chronic disease with disseminated pruriginous nodules from several millimeters up to 2 cm in diameter. It remains unclear whether this disorder is a primary skin disease or results mechanically due to pruritus and scratching provoked by a systemic cause, such as renal insufficiency or an infectious disease (HIV, hepatitis C, etc.). One of the hallmarks of this dermoepidermal disorder, but still controversial, is neuronal hyperplasia in the dermis (Pautrier neuromas).
A 76-year old patient presented in our Departments with disseminated PSS lesions, especially on the arms, which initially improved under local corticosteroids and UVB311 phototherapy. The patient was admitted 1 year later with multiple PNH lesions on the neck, arms, lower extremities and gluteal region. The complete remission of the lesions was achieved after a month of treatment with intralesional triamcinolon injections.

The immunohistochemical analysis of paraffin-compatible neuronal markers (S100, synaptophysin, CD56) was performed on biopsies of both PSS and PNH lesions, as well as healthy perilesional skin. Increased S100+ cells were already observed in the entire epidermis in PSS lesions, which further increased and were documented in the papillary dermis of PNH lesions. Staining against MelanA did not reveal significant differences in melanocyte numbers. In PNH lesions a mild increase of CD56 in the dermis was documented, which was reduced after intralesional steroid treatment. A significant decrease of S100+ cells was documented after intralesional steroid treatment. Synaptophysin staining did not reveal any significant differences between the lesions.
Our data indicate that OSS and PNH are probably different stages of the same pathological entity. Additional number of patients and complementary immunohistochemical data directed against neuropeptides, such as substance P and calcitonin gene related protein are required to corroborate our observation and pathogenetic hypothesis.

P200

Woad plant derived petroleum ether extract mediates anti-inflammatory and anti-allergic effects

T. Lotts[1], C. Miethner[2], M. Schöler[2], K. Agelopoulos[1], I. Schellenberg[2] and S. Ständer[1] [1]*University of Muenster, Center for Chronic Pruritus, Department of Dermatology, Muenster, Germany;* [2]*Anhalt University of Applied Sciences, Center of Life Sciences, Institute of Bioanalytical Sciences (IBAS), Bernburg, Germany*

Allergic contact dermatitis, or atopic eczema, are highly prevalent, itchy dermatoses associated with mast cells, which play a key role in allergic reactions and inflammatory processes. The medicinal herb Isatis tinctoria (woad plant) has demonstrated anti-inflammatory effects on allergen-induced airway inflammation and in acute and subchronic hapten-induced edema models. However, up to now, no defined extract with exact characterization of active substance content were developed. We assume that optimized extracts from the woad plant could alleviate inflammatory skin diseases. Extracts from dried woad leaves by modified petroleum ether extraction (PE) were prepared and analyzed via HPLC with the intention of identifying the constituting and active main compounds (i.e. tryptanthrine, 3-indoleacetonitrile). The resulting PE extracts and a combination of the identified main compounds (MCo) were then tested in different cell culture assays in order to screen for active extracts. The potential toxicity of the PE extracts was analyzed *in vitro* by measuring cell viability in keratinocytes by means of a proliferation XTT assay. Mast cell degranulation experiments, performed by measuring the release of the enzyme beta-hexosaminidase, were used in order to identify the anti-allergic potential of the extracts. The anti-inflammatory activity of the PE extracts was investigated by employing a cell-free COX-2 assay. After screening the extracts in the cell culture, one extract was used on an *in vivo* mouse model with an acute allergic contact dermatitis (contact hypersensitivity [CHS]) induced by 1-Fluoro-2,4- dinitrobenzene (DNFB). Ear swelling was measured 48 h following the challenge. Skin biopsies were taken for histological and molecular biological analysis (HE, Giemsa, immunofluorescence staining, qPCR). We found that low concentrations of the PE extract induced a proliferative effect on keratinocytes (XTT assay), while showing slightly toxic properties at high concentrations. The PE extract inhibited COX-2 activity based on the concentrations, meanwhile exhibiting an IC50 of 8 μg/ml. A concentration-dependent reduction of the substance P and calcium ionophore-induced mast cell degranulation was observed following PE extract and MCo stimulation of mast cells. In all cases, the reduction was significantly different when compared to the untreated control. Significantly reduced ear swelling (PE −63%, $P < 0.001$; MCo −75%, $P < 0.01$) was found in the treated mice of the CHS model compared to the vehicle control group for the PE extract and an equivalent combination of the identified MCo. HE staining confirmed an inhibition of edema formation in the treated ears. Real-time PCR analysis of the ears exhibited a markedly reduced expression of cutaneous interleukin (IL)-1β, IL-4, IL-6 and IL-33, but not of IFN-γ in PE extract-treated murine skin when compared to the vehicle treated group. Giemsa and CD117 staining revealed a 1.5 fold decreased number of mast cells in the skin of the PE extract ($P < 0.01$) and MCo-treated ($P < 0.05$) mice in comparison to the vehicle group. In summary, we have developed a new woad extract that displays anti-inflammatory and anti-allergic properties by inhibiting mast cell responses, COX-2 activity and downregulation of different inflammatory cytokines, and has further proven its efficacy in a mouse model. Our extract could therefore be considered a novel treatment option for contact dermatitis or atopic eczema following demonstration of its beneficial effects in clinical trials.

P201

Characterization of itch perception in inflamed and non-inflamed skin

F. André, T. Hawro, M. Maurer and M. Metz *Charité – Universitätsmedizin Berlin, Dermatology and Allergy, 10117 Berlin, Germany*

Itch is a major symptom of many inflammatory diseases including atopic dermatitis or psoriasis, and impacts greatly on the quality of life in these patients. To explore the specific characteristics of itch in inflamed as compared to non-inflamed skin, we artificially induced itch in normal skin and in experimentally induced eczema in healthy volunteers.
Skin inflammation was induced by sodium lauryl sulfate (SLS 2%) on the volar forearms of 30 healthy volunteers and eczema intensity was assessed using the eczema score adapted from Frosch and Kligman. Non-histaminergic itch was provoked by cowhage spicules rubbed on the volar forearms and recorded for 30 min on a 10 cm visual analogue scale.
Within a week, induction of eczema by SLS resulted in a mild eczema with a score of 2.3 ± 0.09. Cowhage-induced itch was markedly higher in inflamed skin as compared to non-inflamed skin (increase of maximum itch intensity by 61%, $P < 0.0001$), while the overall course of the itch ratings remained similar (i.e. duration and relative intensity over time). The quality of cowhage-induced itch, however, was significantly different between itch in non-inflamed and inflamed skin, with statistically significant more burning and painful sensation in inflamed skin ($P < 0.05$). To further characterize the itch in inflamed skin, we tested for correlation of the maximum itch intensity with the intensity of skin inflammation (eczema severity), age, gender, Erlangen Atopy Score, skin hydration and skin barrier integrity. With the exception of a very weak correlation with eczema intensity ($r = 0.29$), none of the factors showed any correlation. In contrast, itch intensity strongly correlated with maximum itch intensity in non-inflamed skin: Individuals who show a higher maximum itch intensity in noninflamed skin also perceive higher itch in inflamed skin ($r = 0.58$, $P = 0.0004$).
Taken together, itch in inflamed skin is perceived as more intense, painful and burning. Most interestingly, endogenous factors are likely to predispose subjects to experience different intensities of itch. Whether this is associated with differences in skin physiology, neurological or psycho-neurological factors or a combination of all remains to be explored.

P202

Experimental models of induced itch – characterization of tools and methods

T. Hawro, S. Lehmann, E. Deuring, K. Weller, F. André, M. Maurer and M. Metz *Charité – Universitätsmedizin Berlin, Dermatology and Allergy, 10117 Berlin, Germany*

In recent years, the understanding of histamine-independent itch mediators, and their cutaneous and neuronal pathways improved. Establishment of reproducible, experimental models of pruritus, including assessment of local reactions to skin challenge, is crucial for studies on itch perception and for development of potential interventions.
The aim of this study was to compare different methods for assessment of skin flare reaction after provocation with histamine, cowhage and capsaicin and to further characterize these three models of pruritus.
Thirty-one healthy volunteers (15 females, 29.0 ± 4.2 years old) participated in this study (10 atopics). Skin of volar surfaces of forearms was challenged in a randomized order by skin prick testing with histamine and capsaicin and by application of cowhage spicules. As negative controls solutions without active ingredients or deactivated cowhage spicules were used. Skin flare reaction was assessed up to 90 min after challenge using measurement with a ruler, planimetric analysis of digital pictures, assessment of skin temperature using thermoprobe and thermography, laser Doppler flowmetry, laser speckle contrast imaging and colorimetry. Wheal size was measured with a ruler and using volumetric photography. Itch intensity was assessed on a VAS every minute over 30 min after application, and volunteers were asked to assess quality of perceived sensations using selected items from Eppendorf Itch Questionnaire.
Histamine, capsaicin and cowhage induced flare reactions, which were detectable (significantly increased parameters comparing to their negative controls) using colorimetry, laser Doppler imaging and laser speckle contrast analysis. Only intensity of histamine-induced itch correlated with flare. The strongest correlation was observed for the flare recorded using speckle laser contrast imaging, which correlated with histamine-induced itch: duration ($r = 0.61$; $P < 0.001$), maximum intensity ($r = 0.64$; $P < 0.001$) and area under the curve for itch ($r = 0.70$; $P < 0.001$). Weaker correlations of histamine-induced itch parameters were observed also for laser Doppler flowmetry: duration ($r = 0.53$; $P < 0.001$), maximum intensity ($r = 0.47$; $P < 0.001$) and area under the curve ($r = 0.59$; $P < 0.001$). Wheal volume correlated with itch duration after provocation with histamine ($r = 0.66$; $P < 0.001$).
Most pronounced increase in flare parameters was observed for histamine, starting already from the first assessment time-point after skin challenge, for all methods used, with exception of thermography, which failed to document any significant increase in the temperature of skin surface. Capsaicin produced less itching and physical urge to scratch as compared to histamine and cowhage, whereas both capsaicin and cowhage induced more burning than histamine. Cowhage releases more intensive sensations of 'pricking' and 'comes in waves' as compared to histamine. No differences in flare, wheal, itch intensity and perceived qualities of sensations were observed between males and females and between healthy volunteers with and without positive history of atopy.
Histamine, capsaicin and cowhage all induce a flare response, however, flare induced by cowhage requires sensitive analytic techniques, i.e.: laser speckle contrast imaging, laser Doppler flowmetry and colorimetry. Itch induced by histamine correlates with flare assessed with speckle laser contrast imaging and laser Doppler flowmetry. Blood flow in the provocation area achieves its maximum early after provocation and normalizes slowly. Taken together, different itch mediators induce partly different sensations. Using laser speckle contrast imaging enables precise, real-time measurement of flare and its read-outs best correlate with itch intensity for histamine.

Tumor Biology

P203

A distinct role for eosinophil and neutrophil granulocytes in patients with advanced melanoma receiving selective BRAF inhibitors

I. Cosgarea[1,2], C. Franklin[1,2], M. Schwamborn[1,2], A. Sucker[1,2], K. G. Griewank[1,2], B. Weide[2,3], C. Loquai[2,4], J. C. Hassel[2,5], K. C. Kähler[6], J. Utikal[2,7], R. Gutzmer[8], S. M. Goldinger[9], A. Paschen[1,2], D. Schadendorf[1,2] and B. Schilling[1,2] [1]*Department of Dermatology, University Hospital, University Duisburg-Essen, Essen, Germany;* [2]*German Cancer Consortium (DKTK), Heidelberg, Germany;* [3]*University Medical Center, University of Tuebingen, Tuebingen, Germany;* [4]*Department of Dermatology, University of Mainz, Mainz, Germany;* [5]*Department of Dermatology, Heidelberg University Hospital, Heidelberg, Germany;* [6]*Department of Dermatology, Venerology and Allergology, University of Schleswig-Holstein Hospital, Campus Kiel, Kiel, Germany;* [7]*Department of Dermatology, Venereology and Allergology, University Medical Centre Mannheim, Ruprecht-Karl University of Heidelberg, Mannheim, Germany;* [8]*Department of Dermatology and Allergy, Hannover Medical School, Hannover, Germany;* [9]*Department of Dermatology, University Hospital Zürich, Zurich, Switzerland*

Introduction: Selective BRAF inhibitors (BRAFi) such as dabrafenib and vemurafenib prolong survival of patients with advanced melanoma. Selective BRAFi have immunomodulatory effects and murine data indicate that myeloid cells can mediate resistance against these therapeutics. Since no such evidence has been reported in melanoma patients, we performed a translational study to investigate the role of granulocyte subsets in BRAFi-treated melanoma patients.
Materials and Methods: Clinical data of 204 patients receiving selective BRAFi were collected in skin cancer units in Germany and Switzerland. Whole blood counts (WBC) were determined by automated hematology analyzers within 4 weeks prior to treatment. Whole blood underwent gradient centrifugation and human granulocyte subsets were isolated by magnetic cell separation. Primary human melanoma cell lines were generated locally and cultured in RPMI 1640 with 10% fetal calf serum. BRAF status was determined by Sanger sequencing. Tumor cells and granulocytes were co-cultured in the presence of PLX4032 (provided by Plexxikon) or a vehicle control. Cytotoxicity was determined by flow cytometry. Statistical analyses were performed using SPSS.
Results: In a multivariate analysis, low pre-treatment eosinophil counts, high numbers of neutrophils and high levels of LDH were associated with an increased risk for progression (HR 1.38 (95% CI 1.02–1.88), 1.69 (1.24–2.29) and 1.54 (1.21–2.21), respectively) and death (HR 1.40 (95% CI 1.00–1.95), 1.7 (1.21–2.39) and 1.63 (1.13–2.36), respectively) during selective BRAFi treatment. *In vitro*, eosinophils were found to decrease viability of BRAFV600E positive cell lines Ma-Mel 63a, Ma- Mel 51, Ma-Mel 45a and Ma-Mel 11 in a dose dependent manner. Sensitivity of melanoma cells to eosinophil-mediated cytotoxicity was associated with low CD321 expression. In contrast, co-incubation of melanoma cells and neutrophils supported tumor cell survival dose dependently. When Ma-Mel 63a cells were exposed to 0.1 μM PLX4032, eosinophil- but not neutrophil-mediated cytotoxicity was significantly increased. In Ma-Mel 51, incubation with 1.0 μM PLX4032 for 24 h induced apoptosis. However, in the presence of neutrophils but not eosinophils, *in vitro* efficacy of PLX4032 was significantly reduced.
Conclusion: High eosinophil and low neutrophil counts are associated with prolonged survival and response to selective BRAFi in patients with advanced melanoma. Consistently, eosinophils induced apoptosis in melanoma cells while neutrophils supported tumor cell survival *in vitro*. In addition, selective BRAFi seem to enhance eosinophilmediated cytotoxicity while neutrophils might decrease the efficacy of these compounds. Additional studies are needed to unravel the mechanisms underlying this observation and to further clarify the role of myeloid cells in melanoma patients receiving selective BRAFi.

P204 (O04/03)

Histone H2A deubiquitinase 2A-DUB/Mysm1 – a new epigenetic player in skin pigmentation and melanoma growth

I. Banik, C. Wilms, A. Hainzl, C. M. Kröger, M. Wlaschek, K. Scharffetter-Kochanek and M. V. Gatzka *Universitätsklinikum Ulm, Department of Dermatology and Allergic Diseases, 89081 Ulm, Germany*

Histone modifying enzymes, especially components of the polycomb repressive complexes (PRC) such as Bmi1 (PRC1), Ezh2, and Jarid2 (PRC2), are involved in the regulation of melanoma growth, epithelial-mesenchymal transition (EMT) and tumor stem cell maintenance. Comparable with Bmi1 regulating the monoubiquitination of histone H2A at lysine 119 (H2A-K119u), H2A deubiquitinase 2A-DUB/Mysm1 interacts with the p53-axis and other tumor suppressor genes in hematopoeisis and tissue differentiation, in part by modulating DNA-damage responses in stem cell and progenitor compartments. Recently, we demonstrated that loss of Mysm1 aside from other anomalies causes skin atrophy in developing Mysm1tm1a/tm1a (Mysm1−/−) mice in context with decreased proliferation in

epidermis and hair follicles as well as increased apoptosis and accumulation of DNAdamage marker γH2AX in hair follicle stem cells. In the present investigation, we further analyzed the role of 2A-DUB/Mysm1 in melanocyte biology and melanoma formation using a Mysm1-deficient mouse model, human melanoma cell lines and melanoma samples.
Macroscopic anomalies presented as belly spot-and-tail phenotype (white milk spot and skeletal tail deformation) were accompanied by moderately reduced tyrosinase expression/activity in the skin of newborn and young adult Mysm1−/− mice compared with wild-type littermates. In human melanoma cell lines A375 and SK-Mel28 and in cultured human melanocytes, high expression of Mysm1 was detectable on the mRNA and protein level with variable changes upon UV-irradiation. To further explore the function of Mysm1 in melanoma growth, we stably silenced Mysm1 expression in A375 melanoma cells by shRNA-mediated knockdown with GFPcontaining constructs using lentiviral technology. Reduction of Mysm1 expression in A375 cells lentivirally transduced with different Mysm1-shRNA clones was up to 40% of wild-type Mysm1 expression in the surviving cell population. Proliferation of A375 melanoma cells was significantly reduced upon shRNA-mediated knockdown of Mysm1 in both clones obtained. In addition, GFP-positive Mysm1-shRNA A375 cells were more prone to spontaneous apoptosis in culture in comparison with A375 control cells expressing scambled RNA as measured by Annexin V staining and Cell Death ELISA. In line with increased apoptosis, changes in Mcl1 and p19Arf mRNA levels were detectable upon knockdown of Mysm1 in A375 cells. Our *in vitro* melanoma cell growth analyses were complemented by soft-agar assays and by UV-irradiation experiments of Mysm1-deficient mice. In context with our finding that developmental defects in the skin of Mysm1-deficient mice were ameliorated by simultaneous ablation of p53 in Mysm1−/−p53−/− double-deficient mice, this investigation uncovers a potential novel role for histone H2A deubiquitinase 2A-DUB/Mysm1 in regulation of proliferation and survival of melanoma cells with potential implications for tumor therapy.

P205

In vitro approaches to test cold plasma technology as a new treatment option for supportive skin cancer therapy

A. Schmidt, S. Bekeschus, T. von Woedtke and S. Hasse *Leibniz Institute for Plasma Science and Technology, Plasma Life Science, 17489 Greifswald, Germany*

Treatment of tumor progression and metastasis continues to be of major importance in the field of cancer medicine. It is reported that cancer cells often show a pronounced sensitivity towards oxidative stress. Cold plasma technology offers the ability to deliver a delicate mix of reactive oxygen and nitrogen species directly to cells and tissues. The kINPen is a well characterized generator of cold plasma for biomedical applications. It is an argon plasma jet that operates at atmospheric pressure and the generated plasma keeps temperatures below 35°C at working distance. Using the kINPen the aim was to investigate the biological responses of two different skin tumor cell lines regarding cell death, cell migration, and expression of adhesion-associated genes as well as cytoskeletal modifications. We were able to show that plasma-treated medium induced profound effects on tumor cell motility in both, a human melanoma cell line SK-Mel-147 and a head and neck squamous cell carcinoma cell line HNO97. By contrast, changes in cell metabolism, induction of apoptosis, and effects on cell cycle progression were modest. Plasma treatment of cells was associated with an inhibition of migration and disorganization of the actin cytoskeleton which was mediated through multiple signaling pathways, as transcriptome-wide analysis suggested. Specifically, changes in cell adhesion were regulated by differential expression of cell junction and cell-matrix proteins. These results provide evidence that cold plasma technology may be a promising option in support of conventional therapies to disturb the migration and adhesion of skin tumor cells and thus reducing their metastatic activity.

P206

Role of OX40/OX40L and 4-1BB/4-1BBL signaling during cutaneous antitumoral immunity

G. Geidel[1], M. Maurer[2], T. A. Luger[1] and K. Loser[1] [1]*University of Muenster, Department of Dermatology, 48149 Muenster, Germany;* [2]*Charité – Universitätsmedizin, Department of Dermatology and Allergy, 10117 Berlin, Germany*

The receptor ligand pairs OX40/OX40L and 4-1BB/4-1BBL are members of the tumor necrosis factor (TNF) family and it has been shown that signaling via OX40/OX40L and 4 1BB/4-1BBL is critically involved in the control of inflammation and autoimmunity but also regulates anti-microbial or anti-tumoral immunity. Both, OX40 and 4-1BB are expressed on different immune cell subsets and it is known that agonistic antibodies to these co-stimulatory molecules induce T-cell activation. Preclinical data provided the basis for the development of anti-4-1BB and anti- OX40 antibodies as potential therapies for patients with cancer. However, the role of OX40 and 4-1BB signaling during cutaneous anti-tumoral immunity has not been investigated in detail. Therefore, we generated transgenic mice overexpressing OX40 or 4-1BB in the skin (K14-OX40 tg, K14-4-1BB tg) and subcutaneously inoculated them with B16-melanoma cells. Interestingly, K14-OX40 tg and K14-4-1BB tg mice showed a significantly increased tumor growth compared to wildtype (wt) controls. By performing 10-color flow cytometry, immunohistochemistry and gene expression studies we detected increased numbers of CD8 + T cells in melanomas from tg versus wt mice. But these CD8+ T cells showed a similar tumor-specific cytotoxic activity compared to CD8+ T cells purified from wt tumors as evidenced in *in vitro* cytotoxicity assays, suggesting that CD8+ T cells might be of minor importance to explain the increased tumor progression in tg mice. Notably, flow cytometry as well as *in vitro* suppression assays revealed significantly increased levels of myeloid-derived suppressor cells (MDSC) as well as an increased inhibitory function of regulatory T cells (Treg). MDSC and Treg are major components of the immunosuppressive network controlling tumor growth and it has been shown that both, MDSC and Treg, affect the outcome of tumor-associated immune responses by interacting with mast cells. Especially for Treg it is well known that the interaction with mast cells in a tumor environment can be controlled by OX40/OX40L signaling whereas up-regulated 4-1BB signaling expanded mast cells as assessed by the treatment of tumor-bearing mice with an agonistic anti-4-1BB antibody. Therefore, we next quantified the numbers of mast cells in tumor tissue as well as in peripheral blood. Interestingly, the levels of CD117+ mast cells were markedly decreased in melanomas from tg compared to wt mice and low numbers of tryptase+ or chymase+ mast cells have previously been associated with reduced survival in advanced stage melanoma. To assess the role of mast cells during tumor progression in tg mice in more detail we bred K14-OX40 tg mice to KitW-sh mutants lacking CD117+ mast cells. In line with our hypothesis K14-OX40 tg x KitW-sh double mutants showed a markedly increased tumor progression as compared to mast cell competent K14-OX40 tg controls. Thus, our data indicate that up-regulated OX40/OX40L and 4 1BB/4 1BBL signaling in the skin mediated the expansion of MDSC in B16- melanoma-bearing tg mice resulting in the inhibition of tumor-protective mast cells.

P207 (O05/01)

Stat1-induced cancer cell senescence protects from metastases

E. Brenner[1], H. Braumüller[1], T. Wieder[1], K. Schilbach[2], S. Weidemann[1], V. Galinat[1], N. Simon[1], J. Bauer[1] and M. Röcken[1] [1]*Eberhard Karls University, Dermatology, 72076 Tuebingen, Germany;* [2]*Eberhard Karls University, 72076 Tuebingen, Germany*

Metastases are the major cause of death from cancer. Preclinical and clinical data suggest that the interferon and Stat1-signaling pathway play a crucial role in the prevention of metastasis. However, this hypothesis requires formal proof. Importantly it remains unclear whether interferon-mediated protection results from Stat1-signaling in cancer cells or from Stat1-dependent signals in tumor vessels, immune cells or stroma cells. To address these questions, we studied Stat1-proficient and Stat1-deficient (Stat1.ko) mice developing endogenous tumors due to the expression of the simian Virus 40 large T antigen (Tag) the control of the rat insulin promoter (RIPTag2). In sham-treated mice Stat1.ko affected neither growth of primary cancers nor life-time. Yet, while metastases are extremely rare in Stat1-proficient mice (<1%), 44% of RIP-Tag2.Stat1.ko mice developed distant macrometastases. Treatment with tumor-specific, interferon-γ (IFN-γ) and tumor necrosis factor (TNF) producing, tumor-antigen-specific (TAA) T-helper-1 cells (Th1) prevented cancer formation in RIP-Tag2 mice. In contrast, Th1 cells inhibited neither tumor development nor metastases in RIP-Tag2.Stat1.ko mice, demonstrating the critical role of IFN in protecting from metastases. To determine whether Stat1-signaling in the cancer cells was required to protect from metastases, we exposed RIP-Tag2 or RIPTag2. Stat1.ko cancer cell lines to IFN-γ and TNF. *In vitro* treatment with IFN-γ and TNF impaired BrdU-incorporation, arrested the tumor cells in G0/G1, induced to a senescence-like phenotype and increased senescence-associated-β-galactosidase (SA-β-gal) activity only in Stat1-proficient but not in Stat1.ko tumor cells. Likewise, the two cytokines induced a permanent growth arrest only in RIP-Tag2 but not in RIP-Tag2.Stat1.ko cell lines. To determine whether IFN-γ and TNF control the risk of cancer spreading, we injected treated cancer cell lines into NOD-SCID.IL2Rcγ.ko mice. Whereas treated RIP-Tag2 cancers remained growth arrested at ectopic sites, RIP-Tag2.Stat1.ko cancers grew rapidly also after treatment with IFN-γ and TNF. Together, the data show that treatment with Th1 cells or Th1 cell cytokines induced cancer cell senescence and prevented metastatic spreading in a strictly Stat1-dependent manner, even in fully immune compromised mice. In consequence, senescence induction in metastatic cancer cells critically contributes to the long-term arrest of metastatic cancer cells.

P208

Opposing roles of JNK and p38 in lymphangiogenesis in melanoma

E. Puujalka[1], M. Heinz[1], B. Hoesel[2], P. Friedl[1], B. Schweighofer[1], J. Wenzina[1], C. Pirker[3], J. A. Schmid[2], R. Loewe[1], W. Berger[3], E. F. Wagner[4] and P. Petzelbauer[1] [1]*Med Uni Vienna, Dermatology, 1230 Vienna, Austria;* [2]*Med Uni Vienna, Vascular Biology and Thrombosis Research, 1090 Vienna, Austria;* [3]*Med Uni Vienna, Department of Medicine I, 1090 Vienna, Austria;* [4]*CNIO, BBVA Foundation-CNIO Cancer Cell Biology Program, E28029 Madrid, Spain*

In primary melanoma, the amount of Vascular Endothelial Growth Factor C (VEGFC) expression and lymphangiogenesis predicts the probability of metastasis to sentinel nodes, but conditions boosting VEGF-C expression in melanoma are poorly characterized. By comparative mRNA expression analysis of a set of 22 human melanoma cell lines, we found a striking negative correlation between VEGF-C and Microphthalmia-associated Transcription Factor (MITF) expression, which was confirmed by data mining in GEO databases of human melanoma Affymetrix arrays. Moreover, in human patients, high VEGF-C, and low MITF levels in primary melanoma significantly correlated with the chance of metastasis. Pathway analysis disclosed the respective JNK and p38/MAPK activities as being responsible for the inverse regulation of VEGF-C and MITF. Predominant JNK signaling results in a VEGF-Clow/MITFhigh phenotype, these melanoma cells are highly proliferative, show low mobility and are poorly lymphangiogenic. Predominant p38 signaling results in a VEGF-Chigh/MITFlow phenotype, corresponding to a slowly cycling, highly mobile, lymphangiogenic and metastatic melanoma. In conclusion, the relative JNK and p38 activities determine the biological behavior of melanoma. VEGF-C and MITF levels serve as surrogate markers for the respective JNK and p38 activities and may be used to predict the risk of metastasis in primary melanoma.

P209

The PI3K-AKT pathway – a therapeutic target in melanoma brain metastases?

H. Niessner[1], J. Schmitz[2], A. Schmid[2], T. Sinnberg[1], G. Tabatabai[3], B. Weide[1], C. Garbe[1], L. Quintanilla-Fend[4], M. Mai[5], S. Beissert[5], D. Kulms[5], G. Schackert[6], M. Muders[7], M. Meinhardt[7], G. Baretton[7], B. Pichler[2], D. Westphal[5] and F. Meier[1,5] [1]*University of Tuebingen, Department of Dermatology, 72076 Tuebingen, Germany;* [2]*University of Tuebingen, Department of Preclinical Imaging and Radiopharmacy, 72076 Tuebingen, Germany;* [3]*University of Tuebingen, Department of Neurologie, 72076 Tuebingen, Germany;* [4]*University of Tuebingen, Department of Pathology, 72076 Tuebingen, Germany;* [5]*Carl Gustav Carus Medical Center, TU Dresden, Department of Dermatology, Dresden, Germany;* [6]*Carl Gustav Carus Medical Center, TU Dresden, Department of Neurosurgery, Dresden, Germany;* [7]*Carl Gustav Carus Medical Center, TU Dresden, Department of Pathology, Dresden, Germany*

Despite recent advances in treating metastatic melanoma, therapies are less effective in patients with brain metastases than in extracerebral metastases. This highlights the role of the brain environment on tumor progression and drug resistance and the need for 'brain-specific' therapies. We previously showed that the PI3KAKT survival pathway is hyperactivated in melanoma brain but not in extracerebral metastases. In addition, astrocyte-conditioned medium led to activation of AKT in melanoma cells *in vitro*. We thus wanted to investigate the mechanisms of AKT hyperactivation.
The astrocyte-conditioned media experiment suggested that soluble factors secreted by the astrocytes are involved in the increased AKT activation. We continued our studies by investigating the insulin-like growth factor and its receptor (IGF-1R), as the IGF-1R signaling pathway has been shown to activate AKT and has been implicated in playing a role in tumor survival and growth in a variety of human cancers, including melanoma. Indeed, prelimimary immunohistochemical stainigs of brain metastases confirmed the presence of the IGF-1R on the surface of melanoma cells. Further experiments are carried out to correlate the activity of IGF-1R with that of AKT.
In addition, the PI3K inhibitor buparlisib (BKM120) inhibited the phosphorylation of AKT and the growth of >10 newly isolated cell lines derived from melanoma brain metastases achieving growth inhibition rates of up to 80%. These effects did not depend on BRAF, NRAS or KIT mutation status. Furthermore, buparlisib potently induced apoptosis in brain metastatic melanoma cells and significantly inhibited the tumor growth of human BRAF- and NRAS-mutant brain metastatic melanoma cells in the brain of nude mice as shown by MRI scans.
Together these results suggest that melanoma brain metastases might activate different pathways than extracerebral metastases that can be targeted by inhibiting the IGF-1R-PI3K-AKT pathway.

P210

Exogenous induced senescence triggers intrinsic vulnerabilities in pancreatic ß-cell cancer

K. Boehm, H. Braumüller, T. Wieder and M. Röcken *Eberhard Karls University, Department of Dermatology, Tuebingen, Germany*

Cellular senescence plays an important role in tissue development, homeostasis and cancer control. It was known as an intrinsic growth control mechanism that prevents the transformation of pre-malignant lesions into overt malignancy. We recently found in RIP1-Tag2 mice that, in addition to endogenous stress, exogenous signals delivered by the immune system can arrest cancer growth through the induction of cellular senescence in RIP1-Tag2 mice. Thus, in addition to the cancer control by induction of cell death, the immune system can arrest a large spectrum of human and mouse cancer cells by driving them into senescence via the activation of the p16INK4a/Rb pathway in absence of apoptosis. As senescent cancer cells remain a potential harm, we analyzed the molecular changes of cytokine-induced senescence in cancer cells. Such data open up new windows for possible drug targets that allow clearance of senescent cancer cells. In the present study, we found downregulation of CD47, a surface marker that prevents recognition of cells by the immune

system, in senescent β-cells *in vitro*. Further experiments revealed that senescent β-cancer cells were resistant to phagocytosis by bone-marrow derived macrophages *in vitro*. A second signal is needed to induce clearance. Cellular senescence plays an important role in tissue development, homeostasis and cancer control. It was known as an intrinsic growth control mechanism that prevents the transformation of pre-malignant lesions into overt malignancy. We recently found in RIP1-Tag2 mice that, in addition to endogenous stress, exogenous signals delivered by the immune system can arrest cancer growth through the induction of cellular senescence in RIP1-Tag2 mice. Thus, in addition to the cancer control by induction of cell death, the immune system can arrest a large spectrum of human and mouse cancer cells by driving them into senescence via the activation of the p16INK4a/Rb pathway in absence of apoptosis. As senescent cancer cells remain a potential harm, we analyzed the molecular changes of cytokine-induced senescence in cancer cells. Such data open up new windows for possible drug targets that allow clearance of senescent cancer cells. In the present study, we found downregulation of CD47, a surface marker that prevents recognition of cells by the immune system, in senescent β-cells *in vitro*. Further experiments revealed that senescent β-cancer cells were resistant to phagocytosis by bone-marrow derived macrophages *in vitro*. A second signal is needed to induce clearance. Yet, in contrast to the data observed from oncogeneinduced senescence in precancerous cells, cytokine induced senescence strongly increased the susceptibility to secondary apoptosis induced by the kinase inhibitor Staurosporine. Thus, cytokine-induced senescence protects the tumor cells from being recognized and phagocytosed by macrophages, it sensitizes these cells to secondary apoptosis induced by Staurosporine. In consequence, cytokine induced senescence opens a therapeutic window that may allow to selectively clear the potential harmful senescent cancer cells.

P211

Role of SSR2 in melanoma cell biology

B. Garg[1], C. Wagner[1], G. Pathria[1,2], M. Maurer[1] and S. N. Wagner[1] *[1]Medical University of Vienna, Division of Immunology Allergy and Infectious Diseases, Department of Dermatology, 1090 Vienna, Austria; [2]University of Marburg, Biochemical-Pharmacological Center, 35043 Marburg, Germany*

Signal Sequence Receptor 2 (SSR2) was revealed as a possible driver of melanoma metastasis in a subset of patients as exhibited by the systematic search algorithm, INtegrated DEtection of Genomic Outliers (INDEGO). INDEGO is a sequential search across human tumor samples for transcript outlier data points with associated gene copy number variations that are correlated with patient's survival to identify genes with pro-invasive functionality. Encouraged by a successful proof of concept study with validation of MTSS1 as driver of metastasis in human melanoma and the high confidence shown by a Cox Proportional Hazards Model Analysis displaying a statistically significant negative association of SSR2 transcript levels with survival of primary melanoma patients ($P = 0.0098$, HR = 0.115, 95% CI = 0.022–0.593), we hypothesized that SSR2 upregulation could be a driver mechanism in human melanoma.

Utilizing Transwell-Matrigel migration and scratch assays we observed a promigratory role of SSR2 in melanoma cells. Pro-survival effects of SSR2 were examined through FACS-based analysis for induction of apoptosis. SSR2 knockdown led to increased cell death in human melanoma cells and, consistently, increased expression of SSR2 was associated with drug resistance.

Given the established role of SSR2 in protein gating to ER, as a part of the SSR complex, we hypothesized protection against ER stress as a possible mode of action of SSR2. Corroborating our hypothesis, we found a statistically significant gene expression correlation between SSR2 and the transcription factor XBP1 in primary melanoma samples with SSR2 outlier expression. X-Box Binding Protein 1 (XBP1) is induced by stress and the key effector molecule of the IRE1α branch of the Unfolded Protein Response (UPR). Consistent with this hypothesis, we observed that induction of stress in human melanoma cells led to XBP1 upregulation followed by an increase in SSR2 transcript and protein levels.

Together with these data and the fact that transcriptional activity of XBP1s has been shown to have pro-tumorigenic effect, we propose SSR2 as a possible target for abrogation of melanoma.

P212

The impact of BRAFV600i-triggered endoplasmic reticulum stress on apoptosis induction by MEKi in NRAS mutated melanoma cells

H. Niessner[1], I. Wanke[1], C. Praetorius[2], D. Kulms[2], S. Beissert[2], C. Garbe[1] and F. Meier[1,2] *[1]University of Tuebingen, Department of Dermatology, 72076 Tuebingen, Germany; [2]Carl-Gustav-Carus Medical Center, TU Dresden, Department of Dermatology, Tuebingen, Germany*

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

In this study, we investigated whether it is possible to take advantage of ER stress induction to enhance the antitumor activity of MEK inhibitors in patients with NRASmutant melanoma.

BRAF-mutant and NRAS-mutant metastatic melanoma cell lines were treated with the BRAF inhibitors vemurafenib, dabrafenib and encorafenib and were subjected to electron microscopy. All of the three substances were able to induce morphological features of ER stress, including a significant dilation of the ER in both BRAF-mutant and NRAS-mutant melanoma cell lines. As expected, the BRAF inhibitors inhibited the phosphorylation of ERK and growth inhibition and induced apoptosis in BRAFmutant but not in NRAS-mutant melanoma cells in monolayer and spheroid culture. However, the BRAF inhibitors significantly enhanced growth inhibition and apoptosis induced by the MEK inhibitors. Moreover, the expression of the ER stress-related factors p8, ATF4, ATF3 and CHOP was induced. siRNA inhibition of ATF4 reduced melanoma cell apoptosis induced by the combinations.

These data suggest that BRAFV600 inhibitors induce endoplasmic reticulum stress and potentiate the antitumor activity of MEK inhibitors in NRAS-mutant melanoma.

P213

Influence of p53 family member activity on therapy resistance in malignant melanoma

E. Makino, T. Sinnberg and B. Schittek *University of Tuebingen, Department of Dermatology, Dermatooncology, 72076 Tuebingen, Germany*

The long-term efficacy of BRAFV600E and MEK inhibitors in metastatic melanoma therapy is limited due to the evolution of different resistance mechanisms. In this study we evaluated the effects of the BRAFV600E inhibitor vemurafenib and MEK inhibitor trametinib on the activation of p53 and different p73 isoforms as well as its relevance on cell cycle arrest and apoptosis induction in melanoma cells.

We could show that cells with wild type p53 and BRAFV600E activate p53 upon BRAF inhibitor treatment. Additional p53 activation by co-treatment with nutlin-3, PRIMA-1 as well as the treatment with the chemotherapeutic agent cisplatin strongly enhances the cytotoxic effects of MAPK inhibitor treatment in sensitive and vemurafenib-resistance acquired melanoma cells. In line with this, overexpression of wild type p53 achieves a similar effect to vemurafenib therapy in p53 mutated melanoma cell lines which additionally influences the expression of different p73 isoforms. We further found a correlation between the potential of p53 activation and Mdm2 expression among the analyzed metastatic melanoma cell lines. Interestingly, MAPK inhibitor resistant cell lines elicit elevated sensitivity to cisplatin in comparison to the sensitive parental cell lines. G2-phase arrest as well as apoptosis induction by cisplatin treatment occur in a higher extend in the resistance acquired cells than in the parental sensitive cells via the enhanced activation of growth arrest related or pro-apoptotic p53 targets. In addition, cisplatin treatment alters the expression of DNp73 in the sensitive and resistant melanoma cells.

Our results propose a p53 family members dependent effect of BRAF and MEK inhibitors treatment. Further experiments are needed in order to evaluate the role of different p53 family member isoforms on BRAF- and MEK inhibitors efficacy and resistance development.

P214

MITF regulates cell adhesion and subcompartment-specific distribution of differentially cycling melanoma cells

C. A. Tonnessen[1], K. A. Beaumont[2,3], L. Spoerri[1], D. S. Hill[2,4], R. J. Jurek[5], S. M. Daignault[1], F. Ahmed[1], M. Fane[6], A. Smith[6], W. Weninger[2,7] and N. K. Haass[1,7] *[1]The University of Queensland, The University of Queensland Diamantina Institute, Translational Research Institute, 4169 Brisbane, Australia; [2]The Centenary Institute, 2042 Sydney, Australia; [3]University of Sydney, Sydney Medical School, 2006 Sydney, Australia; [4]Newcastle University, Dermatological Sciences, NE2 4HH Newcastle upon Tyne, UK; [5]CSIRO Astronomy & Space Sciences, 2008 Sydney, Australia; [6]University of Queensland, School of Biomedical Sciences, 4072 Brisbane, Australia; [7]University of Sydney, Discipline of Dermatology, 2006 Sydney, Australia*

Melanoma drug resistance may be due, in part, to dynamic heterogeneity. Cancer cells within a tumor exhibit various phenotypes in response to environmental stress. This results in populations with different proliferative and invasive capabilities and drug sensitivities. Understanding the molecular signature of dynamic heterogeneity is crucial to design more effective therapies.

Using the fluorescence ubiquitination cell cycle indicator (FUCCI) system, which delineates the cell cycle phases by visual means, we found two phenotypic cohorts of xenografts: One contained distinct clusters of either arrested or proliferating cells and another displayed a homogenous dispersion of proliferating cells throughout. The cohorts expressed either low or high levels of microphthalmia-associated transcription factor (MITF), respectively. Silencing MITF by shRNA converted the phenotype. In a 3D spheroid model, MITF was predominantly expressed in the periphery of the spheroid, which corresponded with the region of highly proliferative cells. Forced over-expression of MITF resulted in loss of a distinct proliferative zone, and instead a homogenous growth pattern. Not only do spheroids express MITF around the perimeter, but also markers of the Epithelial to Mesenchymal Transition (EMT), such as Vimentin and Slug, which upon MITF overexpression also switch to become expressed homogenously. Surprisingly, the increased levels of EMT marker expression by MITF do not correlate to increased migration, and these spheroids in fact show reduced invasion into collagen. Here we show, that this is due to altered cell-cell and cell-matrix adhesion.

These data outline how dynamic heterogeneity, including proliferative and invasive potential, is tightly intertwined with MITF expression, making it an important marker for therapy design.

P215

Targeting of a minor drug-resistant melanoma subpopulation expressing the B cell marker CD20

M. Maurer[1], R. Somasundaram[2], K. L. Bennett[3], M. Perego[2], C. Wagner[1], C. Brüggen[1], J. Griss[1], K. Garg[1], F. Breitwieser[3], J. Colinge[3], A. C. Müller[3], H. Esterbauer[4], M. Herlyn[2] and S. N. Wagner[1] *[1]Medical University of Vienna, Department of Dermatology, Division of Immunology, Allergy and Infectious Diseases, 1090 Vienna, Austria; [2]The Wistar Institute, Philadelphia, USA; [3]CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, 1090 Vienna, Austria; [4]Medical University of Vienna, Department of Laboratory Medicine, 1090 Vienna, Austria*

Cancer cell subpopulations with tumor-initiating or tumor-maintaining properties are able to survive chemo- and/or targeted therapies and thus can contribute to cancer re-growth and relapse. In melanoma, recent therapeutic achievements including the use of BRAF/MEK inhibitors (BRAFi/MEKi) are counteracted by the frequent emergence of drug-resistance followed by recurrence of the disease even after initial responses. Tumor subpopulations have also been identified in human melanoma, including one expressing the B cell marker CD20. Based on the observation that CD20+ melanoma cells follow the definition of tumor-initating cells we now hypothesized that this subpopulation may be able to escape therapy via increased drug-resistance and thus contributes to tumor recurrence.

Expression of CD20 on human melanoma cells leads to increased resistance against chemotherapy and targeted therapies (BRAFi/MEKi) in 2D as well as in 3D melanoma cell culture models. In addition, patient derived xenografts (RPDX) generated from treatment (BRAFi)-resistant BRAFV600E melanoma metastases expressed increased levels of CD20. When we further treated chemoresistant CD20+ human melanoma cells with an anti-CD20 antibody we observed induction of apoptosis *in vitro*. In xenotransplantation assays onto NOD/SCID/c−/− mice systemic administration of an anti-CD20 antibody significantly reduced *in vivo* tumor growth of chemoresistant CD20+ human melanoma cells in adjuvant and therapeutic settings.

In order to identify a molecular mechanism for CD20-mediated drug resistance we performed proteomic profiling of matched CD20− and CD20+ human melanoma cells. These data suggested a resistance mechanism characterized by hyperactivation of MEK/ERK signaling and increased resistance to apoptosis. Validation experiments are ongoing to prove the contribution/requirement of these pathways and to identify the responsible molecular players for CD20-mediated drug-resistance.

Together these data provide direct experimental evidence for a linkage of drug-resistance with the CD20+ phenotype of human melanoma cells and for the contribution of this subpopulation to *in vivo* tumor growth. We believe that such data may hold the potential for a paradigm-shift in cancer treatment: in addition to targeting the bulk of cancer cells, specific targeting of (a) minor cancer subpopulation(s) may be required to fully eradicate established disease and/or to prevent recurrence of the disease.

P216

CEACAM1: a novel target of MITF in malignant melanoma

S. Löffek[1,2], N. Ullrich[1,2], S. Horn[1,2], A. Görgens[3], I. Davidson[4], B. B. Singer[5], D. Schadendorf[1,2], C. R. Goding[6] and I. Helfrich[1,2] *[1]Skin Cancer Unit of the Dermatology Department, Medical Faculty, 45147 Essen, Germany; [2]West German Cancer Center, University Duisburg-Essen, 45147 Essen, Germany; [3]Institute for Transfusion Medicin, Medical Faculty, 45147 Essen, Germany; [4]Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France; [5]Institute of Anatomy, Medical Faculty, 45147 Essen, Germany; [6]Nuffield Department of Clinical Medicine, Ludwig Institute for Cancer Research, University of Oxford, Oxford, UK*

The carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is a widely expressed multi-functional cell-cell adhesion protein, which appears mainly as four isoforms differing in the length of their extra- and intracellular parts. Recently, we provided substantial *in vitro* and *in vivo* evidence for the differential impact on melanoma progression and immune-surveillance in an isoform-specific mode of action and identified CEACAM1-4L as a major driver in these processes.

In order to gain insights into the molecular mechanism we analyzed the ability of cells expressing different CEACAM1 isoforms to form colonies in a soft agar assay. Enhanced expression particularly of the splice variant CEACAM1-4L supports an anchorage-independent signature in melanoma cells. Interestingly, secretome analysis revealed distinct and significant changes in the expression of soluble factors associated with MMP expression and activation (including uPAR, IL-6, EMMPRIN and RANTES) especially in the CEACAM1-4L expressing melanoma cell transfectants. Indeed, as the addition of MMP-specific inhibitors interfered with anchorage-independent growth, we conclude that MMPs (in particular MMP-2) are crucially involved in CEACAM1-4L-induced anchorage-independent growth. Based on the significant role of CEACAM1 expression in malignant melanoma, we were consequently interested in exploring potential regulatory mechanisms. We identified a novel role for the master regulator of melanocyte differentiation and melanoma oncogene MITF as a direct regulator of CEACAM1 expression in melanoma cell lines and tissue. Moreover, Cancer Genome Atlas (TCGA) database-based analyses revealed significant correlation of MITF and CEACAM1 expression in patient-derived melanoma tissues.

Taken together, these novel mechanistic insights into CEACAM1 function as well as the regulation of its expression might help to decipher new targets for the development of innovative therapeutic strategies for the treatment of malignant melanoma.

P217

Targeted combination therapy for BRAF/MEK inhibitor-resistant melanoma cells

A. Hamel, C. Kammerbauer, S. A. Graf and C. Berking *Ludwig-Maximilian University, Department of Dermatology and Allergy, 80337 Munich, Germany*

Treatment of metastatic melanoma has evolved substantially during the last decade. Approximately 50% of all cutaneous melanomas harbor a mutation in the BRAF kinase at position V600, a mediator in the mitogen activated protein kinase (MAPK) signaling pathway. Therapeutic strategies have therefore concentrated on targeted treatments to block different members of this pathway. The inhibition of BRAF kinase with the specific inhibitors (BRAFi) vemurafenib or dabrafenib showed till then unprecedented results. The combination of a BRAF- with a MEK-inhibitor (MEKi), such as trametinib or cobimetinib, has even achieved higher overall response and survival rates than BRAFi monotherapy and has recently been clinically approved in Europe. However, resistance to therapy is still only delayed but not abrogated. This is why our study aims to find an effective third inhibitor for a combination therapy especially for BRAFi/MEKi-resistant melanoma cells.

We generated different BRAFi- and BRAFi/MEKi-resistant melanoma cell lines from established and freshly isolated melanoma tissue cultures by chronic exposure to vemurafenib and selumetinib or trametinib. All of the analyzed BRAFi- and BRAFi/MEKi-resistant cells showed reactivation of the MAPK signaling pathway. Furthermore, BRAFi-resistant WM239a melanoma cells showed an increase in the phosphoinositide 3-kinase (PI3K) signaling pathway.

We investigated cell viability in parental cells, vemurafenib-resistant cells, and vemurafenib- plus trametinib- or selumetinib-resistant cells upon exposure with triple inhibitor combinations including PI3K inhibitor BKM-120, ERK inhibitor GDC-0994, and pan fibroblast growth factor receptor (FGFR) inhibitor BGJ-398.

Decreased viability was detected in parental WM239a cells upon treatment with BKM-120 and GDC-0994, but not with BGJ-398, respectively. Generally, the triple combination of vemurafenib, selumetinib and BKM-120, GDC-0994, or BGJ-398 showed best response rates in all cell lines. Cell viability of BRAFi- and BRAFi/MEKi-resistant cells was less affected upon treatment with GDC-0994 compared to parental cells. BKM-120 treatment was more effective in single and in combinational treatment compared to GDC-0994 therapy in all cell lines. Strikingly, BGJ-398 effectively decreased viability of BRAFi-resistant and particularly of BRAFi/MEKiresistant cells, while parental cells were hardly affected. The combination of BGJ-398 with vemurafenib and/or selumetinib led to almost complete cell death.

Upon BGJ-398 treatment, BRAFi-resistant and especially BRAFi/MEKi-resistant cells showed, a decreased activation of ERK and AKT, which means a downregulation of the MAPK signaling pathway, a reduction of cell cycle regulators and hence cell cycle arrest, as well as activated caspase 3 indicating apoptosis.

FACS analysis of BRAFi- and BRAFi/MEKi resistant cell lines generally showed increased rates of cell death and in particular of apoptotic cells under treatment with BGJ-398 alone and in combination with vemurafenib and selumetinib compared to parental cell lines.

To analyze cell invasion, we established a 3D-spheroid cell culture model. Both BRAFi- and BRAFi/MEKi- resistant cells displayed earlier and stronger invasion than parental cells. Under treatment with BGJ-398 alone and in combination with vemurafenib and selumetinib, the resistant cell lines almost completely lost their capacity to migrate.

These results will be further investigated in more sophisticated *in vitro* models and additional cell lines. Moreover we will examine the molecular mechanisms in BRAFi/MEKi-resistant cells that lead to this high sensitivity to BGJ-398.

In summary, our data provide evidence for FGFR as a promising target for future therapeutic strategies in BRAFi/MEKi-resistant melanomas.

P218

A functional type I IFN system in myeloid immune cells is required for effective adoptive T cell immunotherapy of melanoma

D. A. Lopez Ramos[1], T. Bald[1], M. Hölzel[2] and T. Tüting[1] *[1]University of Bonn, Laboratory of Experimental Dermatology, 53127 Bonn, Germany; [2]University of Bonn, Institute of Clinical Chemistry and Clinical Pharmacology, 53127 Bonn, Germany*

Background: Metastatic melanoma is a deadly disease and has remained a therapeutic challenge for the past decades. It is well known that primary and metastatic melanomas in different patients show considerable variability in the extent of tumor-infiltrating lymphocytes. The underlying mechanisms that regulate the recruitment and function of immune cells in the tumor microenvironment are poorly understood. Evidence is accumulating that primary and metastatic melanomas with T cell infiltrates and a type I IFN signature have a better overall prognosis. On this basis, we hypothesized that type I IFN system is required for optimal efficacy of adoptive T cell immunotherapy for melanoma.

Methods: To experimentally test this hypothesis we treated established HCmel3 melanomas in global and conditional Ifnar1-deficient and -competent C57BL/6 mice with adoptively transferred melanoma specific CD8 T cells. Tumor growth kinetics, overall survival, T cell expansion and effector function as well as tumor-infiltrating immune cells were analyzed.

Results: Adoptive T cell transfer therapy failed to control melanomas transplanted in Ifnar1-deficient mice. Surprisingly, we observed significantly elevated numbers of T cells in the blood of Ifnar1-deficient compared to -competent mice. Histological analysis showed loss of gp100 in melanoma cells that escaped immunesurveillance in Ifnar1-deficient mice associated with a proinflammatory tumor microenvironment mostly composed of myeloid immune cells. Interestingly, increased T cell expansion and early escape due to inflammation-induced dedifferentiation were recapitulated in conditional knockout mice lacking a functional type I IFN system only in myeloid immune cells. Additionally, hypoxia, inflammation and dedifferentiation signatures were prevalent in global gene expression analysis of tumor samples from Ifnar1- competent and -deficient mice. Moreover, immunohistochemical analysis of melanomas showed higher Glut1 and hypoxyprobe-1 stained areas in Ifnar1- deficient compared to -competent mice. In line with the histological analysis, *in vitro* experiments showed that HCmel3 cells lose antigen expression under hypoxic conditions and that myeloperoxidase activity in neutrophils is limited by the type I IFN system.

Conclusions: Taken together, our results show that type I IFN signaling in myeloid immune cells controls the balance between immunity and inflammation in melanoma and is required to regulate T cell expansion, the differentiation state of melanoma cells and their resistance to therapy.

P219

Interfering with stem cell-specific gatekeeper mechanisms results in skin tumour initiation and progression

K. Reuter and C. Niemann *University of Cologne, Center for Biochemistry II and Center for Molecular Medicine Cologne, 50931 Cologne, Germany*

Mammalian skin is constantly assaulted by genotoxic stress such as UV irradiation. Recent studies have shown that epidermal stem cells (SCs), which are crucial for maintaining skin homeostasis, respond differently to stress and DNA damage compared to their rapidly cycling progeny. In particular, multipotent hair follicle SCs are more resistant to DNA-damage-induced cell death than other cells of the epidermis. This has been linked to a higher expression of the pro-survival factor Bcl2 and attenuated p53 activation as a consequence of faster but error-prone DNA repair activity. The relevance of these SC-specific gatekeeper functions for the process of skin tumour initiation has not been investigated. Here, a mutant form of the transcription factor Lef1, mimicking mutations found in human sebaceous tumours, was expressed specifically within the HF bulge SC compartment. Interestingly, targeted expression of mutant Lef1 results in SC-driven sebaceous tumour formation, supporting recent lineage tracing experiments, which identified HF bulge SCs as a cell-of-origin for skin tumours. Mechanistically, mutant Lef1 induces DNA damage and interferes with SC-specific functions normally protecting against accumulations of DNA lesions and cell loss. In particular, mutant Lef1 blocks the Bcl2 response in HF bulge SCs and increased DNA damage induces apoptosis. To compensate the loss of stem cells und to guarantee tissue maintenance, proliferation was stimulated within the SC compartment. This resulted in propagation of cells that escape normal cell cycle control, thereby supporting the accumulation of tumour-initiating mutations. Furthermore, mutant Lef1 disturbs p53 response by antagonizing ATMChk2 dependent stabilization of p53. Interestingly, manipulating p53 levels in SCdriven sebaceous tumours, revealed a new function of p53, determining skin tumour growth, differentiation and immune cell infiltration. Thus our data demonstrate that normal SC regulation is disrupted by mutant Lef1, representing a new mechanism of tumour initiating events in tissue SCs and showing the importance of a tight control of these crucial SC-specific surveillance mechanisms to prevent tumourigenesis. In addition, our results demonstrate for the first time a functional link of p53 levels with the differentiation of epidermal tumours *in vivo*, suggesting p53 as diagnostic marker for different types of sebaceous tumours.

P220

In and outside the melanoma cell: the Y-box binding protein 1 as a novel tumour marker and therapeutic target in vemurafenib resistance

C. Kosnopfel[1], T. Sinnberg[1], A. Münchow[1], A. Schmitt[2] and B. Schittek[1] *[1]University of Tuebingen, Department of Dermatology, Division of Dermatooncology, 72076 Tuebingen, Germany; [2]Interfaculty Institute of Biochemistry, Tuebingen, 72076 Tuebingen, Germany*

The Y-box binding protein 1 (YB-1) is a multifunctional protein involved in various cellular processes including both transcriptional and translational regulation of target gene expression. Significantly increased intracellular YB-1 levels have been reported in a number of human malignancies and shown to be associated with poor prognosis and disease recurrence. Our previous data indicated that YB-1 is upregulated and translocated to the nucleus during melanoma progression and that YB-1 plays an important role in the regulation of proliferation, survival and invasive growth of metastatic melanoma cells.

We can now show, that the S102-phosphorylation as well as the nuclear activity of YB-1 is significantly enhanced in melanoma cell lines with acquired resistance to vemurafenib. This increased YB-1 activation is based on elevated MAPK signalling and seems to be mediated by the active p90 ribosomal S6 kinase (RSK) signalling. Intriguingly, both RSK inhibition and downregulation of total YB-1 levels can increase the sensitivity of vemurafenib resistant melanoma cell lines to PLX4032 treatment.

Next to its intracellular function, we found YB-1 to be strongly secreted by melanoma cells as opposed to benign cells of the skin (e.g. melanocytes, keratinocytes and fibroblasts), which interestingly seems to correlate with the stage of melanoma progression. Based on previous findings postulating a mitogenic function of extracellular YB-1 in both an inflammatory and a breast cancer setting, the functional effects of secreted YB-1 in terms of malignancy as well as its potential role as a melanoma marker were further analysed in this study.

In summary, these data suggest that active RSK signalling mediates YB-1 Ser102- phosphorylation, which might be an attractive therapeutic target in melanoma cells to overcome vemurafenib resistance, while at the same time, extracellular YB-1 secreted by melanoma cells may serve as a novel tumour marker.

P221

Oncogenic role of miR-150 in melanoma

A. Bhattacharya[1,2], U. Schmitz[3], S. K. Gupta[4], J. C. Simon[1] and M. Kunz[1] *[1]University of Leipzig, Department of Dermatology, Venereology and Allergology, 04103 Leipzig, Germany; [2]Charité-Universitätsmedizin Berlin, Molekulares Krebsforschungszentrum, 13353 Berlin, Germany; [3]University of Sydney, Centenary Institute, 2042 Newtown, Australia; [4]University of Rostock, Department of Systems Biology and Bioinformatics, 18051 Rostock, Germany*

Melanoma is one of the most aggressive forms of skin cancer and is highly treatment resistant in the metastatic stage. The understanding of the complex molecular regulation of gene expression at different melanoma stages by non-coding RNAs including microRNAs remains insufficient. We performed a quantitative real-time PCR based miRNA expression profiling involving tissue specimens from primary melanomas and melanoma metastases and compared the expression profiles with those of primary melanocytes. The most significantly upregulated candidate miRNAs during tumor development and progression (miR-126, -142, -150, -214, -221, -345 and -93) were tested for their growth-promoting potential in an *in vitro* clonogenic assay in four different melanoma cell lines (three human and one mouse). Importantly, miR-150, miR-93 and miR-345 significantly enhanced the clonogenic growth of at least three of these cell lines including the mouse melanoma cells. In a mouse melanoma model, syngeneic mouse melanoma cells overexpressing miR-150 formed significantly larger tumors than cells overexpressing both other miRNAs and control cells. Furthermore, knockdown of miR-150 in mouse melanoma cells resulted in attenuated tumor growth as compared to controls. Melanoma cells overexpressing miR-150 indeed showed augmented growth properties and demonstrated compromised levels of tumor suppressor protein p53 (TP53). Although knockdown of miR-150 increased p53 expression and thereby resulted in acute growth arrest, there was no evidence of apoptosis induction (no cleavage of pro-caspase 3). Importantly, miR-150 also suppressed the expression of tumor suppressors CDKN1B (p27) and CDKN2A (p16). Both are important markers for cellular senescence. These data were suggestive of an apoptosis-independent function of the miR-150 interaction with classical tumor suppressor genes which needs further investigation. In conclusion, our study identified microRNAs as upstream regulators of molecular events leading to melanoma formation which may also re-program melanoma cells for metastasis.

P222 (O06/06)

Loss of CYLD promotes melanoma progression

M. M. de Jel, S. Schiffner, S. Kuphal and A. K. Bosserhoff *Institute of Biochemistry, Biochemistry and Molecular Medicine, 91054 Erlangen, Germany*

Depletion or mutation of CYLD have been shown to be associated with development and progression of a variety of cancers including breast cancer, renal cell carcinoma, colon cancer and also malignant melanoma. Our group investigates the tumor suppressive role of CYLD in malignant melanoma which represents the most aggressive form of skin cancer with incidence rates increasing still. We were able to show that expression of CYLD is down-regulated in consequence of increased expression of the transcription factor Snail1. The repression of CYLD results in increased proliferation and invasion of human melanoma cells.

To study the effect of CYLD on melanoma tumorigenesis *in vivo*, CYLD knockout mice were crossed with Tg(Grm1) EPv mice that develop melanoma spontaneously. Protein data showed that the CYLD level is reduced in tumor samples compared with nevi in the transgenic Tg(Grm1) EPv mice and indeed lost in the Tg(Grm1) EPv Cyld−/− mice. Moreover, analyses of this mouse model exhibited that Cyld-deficient mice develop melanoma significantly earlier and show an increased tumor growth compared to the Grm1 control group.

In order to characterize mechanisms through which CYLD mediates its tumor suppressor function, we generated primary and metastatic tumor cell lines of the transgenic mice and performed functional assays. These *in vitro* assays revealed that loss of CYLD leads to an elevated migratory and proliferative potential as well as an enhanced colony formation. The metastatic spread of malignant melanomas occurs primarily through lymph nodes. Using tube formation assays, immunofluorescence staining for the lymphatic vessel marker LYVE-1 and an angiogenesis kit for protein analyses, we showed that loss of CYLD causes an augmented potential for the formation of new blood and lymphatic vessels in murine cell lines and tissues.

Altogether, these findings reveal that loss of CYLD leads to an earlier melanoma onset as well as an accelerated melanoma growth *in vivo*. In addition, *in vitro* assays indicate that loss of CYLD causes an enhanced ability to form colonies from single cells and improves metastatic features through promotion of (lymph-) angiogenesis in murine melanoma cell lines.

P223

Real-time cell cycle and cell death imaging of the effect of sphingosine kinase inhibition on 3D melanoma spheroids

A. Kienzle[1,2], E. McGowan[3,4], G. Douglas[3], D. Kaczorowski[3], W. Weninger[4,5] and N. K. Haass[2,4] *[1]Department of Dermatology, University of Mainz, 55101 Mainz, Germany; [2]The University of Queensland Diamantina Institute, Translational Research Institute, 4102 Brisbane, Australia; [3]Medical and Molecular Biosciences, University of Technology Sydney, 2007 Sydney, Australia; [4]The Centenary Institute, 2042 Sydney, Australia; [5]Discipline of Dermatology, University of Sydney, 2006 Sydney, Australia*

Aims: Inhibition of sphingosine kinase 1 (SK1) is a promising novel approach for treatment of melanoma, a cancer known to be highly resistant to conventional treatment. SK1 plays a critical role in determining the dynamic balance between the proapoptotic sphingolipid metabolite ceramide and the prosurvival sphingosine-1- phosphate By utilising fluorescent ubiquitination-based cell cycle indicator (FUCCI) 3D melanoma spheroids and DRAQ7 staining, we examined the impact of modification of the SK1 pathway on the cell cycle and cell death as a novel therapeutic approach.

Methods: Microarray, qPCR, Western blot, enzyme activity assay, cell proliferation assay, cell cycle flow analysis, migration assays, 3D spheroid melanoma model, FUCCI.

Preclinical *in vitro* studies often poorly predict the outcome of clinical studies. Thus, we use a novel cell culture model which provides a closer comparison to the *in vivo* situation: melanoma cells grown as 3D spheroids mimic tumour architecture and microenvironment better. Here we discuss the anti-melanoma activity of the modification of the SK1 pathway in our 3D spheroid models. The use of the FUCCI system allowed us to conduct real-time cell cycle analysis in both, conventional 2D assays and 3D spheroids. Cell death was analysed in real-time using DRAQ7 staining.

Results: The presence of SK1 in 29 investigated melanoma cell lines was confirmed by microarray, qPCR and Western blotting, albeit at varying expression levels. The functionality of SK1, regardless of the expression level, was proven by enzyme activity assays. Significant melanoma growth reduction was achieved through modification of the SK1 pathway by inhibition of different stations within the pathway. Fingolimod (FTY720), dimethyl-sphingosine (DMS) and sphingosine kinase inhibitors 1 and 2 (SKI-1, SKI-2) decreased cell viability and cause cytotoxicity in all investigated melanoma cell lines. Interestingly, whereas DMS and SKI-1 caused G1-phase cell cycle arrest, FTY720 and SKI-2 caused G2-phase cell cycle arrest. However, in neither case the arrest was as profound as in the positive G1-arrest control (MEK inhibitor U0126), indicating that cell death in the SK-inhibited cells is primarily not dependent on a specific cell cycle phase.

Conclusions: Utilising real-time cell cycle and cell death imaging we show here that modification of the sphingosine kinase pathway has cytostatic and cytotoxic effects on melanoma in 2D and 3D. This is important as different subpopulations of 3D spheroids and *in vivo* melanoma tumours show different cell cycle behaviour and respond differently to drugs. When using conventional methods the effect of Sphingosine Kinase inhibition would appear superficially very similar to that of MEK inhibition, our model allows the investigation of subtle differences in mechanism of action in real time and in 3D.

P224

ADAM-9 modulates melanoma development and metastasis *in vivo*

N. Giebeler[1], A. Schönefuß[1], J. Landsberg[2], T. Tüting[2], C. Mauch[1] and P. Zigrino[1] *[1]University of Cologne, Department of Dermatology and Venerology, Cologne, Germany; [2]University of Bonn, Department of Dermatology and Allergology, Bonn, Germany*

In previous studies we observed increased expression of ADAM-9 in melanoma, in both tumor and stromal cells of the tumor-stroma interface. To further characterize the role of ADAM-9 in melanoma *in vivo*, we have generated mice which are deficient for ADAM-9 and carry the transgene and knock-in mutation Hgf/Cdk4, these last known to spontaneously develop melanoma closely resembling human tumors. These animals were either followed over time for spontaneous melanoma formation or treated with DMBA to induce tumors with faster kinetic. Upon DMBA treatment, mice lacking ADAM-9 developed a higher number of tumors at early time point while, at later time points the number was significantly lower as compared to control mice. At the age of 1 year the numbers of spontaneous tumors developed in Adam-9−/−/Hgf/Cdk4 mice were also significantly reduced as compared to control mice, thus indicating that this effect was not dependent on the DMBA induction. Detailed analysis of the tissues showed that altered proliferation of the tumor cells, but not apoptosis and inflammation, may be responsible for the different tumor development. Importantly, deletion of ADAM-9 resulted in significantly reduced lung metastases post DMBA treatment. This effect, even though less prominent, was also detected in untreated Adam-9−/−/Hgf/Cdk4 mice as compared to controls at ca. 1 year of age.

In Adam-9−/−/Hgf/Cdk4 mice, intravasation of melanoma cells was not impaired as we found equal amount of circulating melanoma cells in the blood of DMBA-induced tumor bearing mice of both genotypes. However, *in vitro* transmigration of melanoma cells through the endothelium was impaired in ADAM-9 deficient melanoma cells. Furthermore, depletion of ADAM-9 in melanoma cells resulted in their decreased invasion in de-epidermized human skin composites. This effect could be rescued by supplying soluble ADAM-9 to the system. Taken together, these data show that ADAM-9 *in vivo* modulates melanoma development and metastatic potential in an induced and spontaneous model of melanoma, and that this activity may be relevant for the human system.

P225

XIAP down regulation inhibits invasion of melanoma cells by regulating cell migration and survival

O. Ayachi[1], M. Barlin[1], S. Bozorg Nia[2], H. Kashkar[2], P. Zigrino[1] and C. Mauch[1] *[1]University of Cologne, Dermatology and Venerology, Cologne, Germany; [2]University of Cologne, Institute for Medical Microbiology, Cologne, Germany*

Apoptotic cell death is a natural characteristic of living systems and it is tightly regulated at different molecular levels to ensure correct development. Known regulators are the inhibitor of apoptosis proteins (IAPs). Expression of c-IAP1, c- IAP2, and XIAP is significantly increased in several cancers including melanoma, and their activity is intracellularly regulated by endogenous inhibitors such as Smac. *In vitro*, XIAP and Smac are expressed in melanoma cell lines of high invasive grade, MeWo, A375 and BLM cells (with higher expression in A375 and BLM). All cells were able to efficiently invade dermal skin equivalents *in vitro*. Inhibition of IAP using Smac mimetics led to a significant inhibition of invasion that was stronger in BLM as compared to MeWo and A375 cells. To address further the role of XIAP expression for BLM invasive abilities, we have stably silenced XIAP expression in these cells (sh-XIAP BLM). XIAP down-regulation did not affect expression of the other cIAPs, indicating that no compensation by other molecules occurred. Moreover, we detected a significantly reduced proliferation in sh-XIAP BLM as compared to control clones, but not altered rate of apoptosis. However, when sh-XIAP cells were stimulated with TRAIL they underwent apoptosis to a higher extent than control cells. In addition, treatment prior to TRAIL stimulation with the necroptotic inhibitor nec-1 or the caspase inhibitor z-vad, revealed that in BLM cells lacking XIAP necroptosis via a RIPK1 dependent pathway is induced.

Interestingly, migration of sh-XIAP BLM cells on fibronectin coated surfaces was significantly reduced likely as consequence of reduced cellular organization on this substrate. Indeed, in contrast to controls, sh-XIAP BLM cells failed to organize their actin filament network and to localize vinculin at cellular borders when plated on fibronectin. As result from all these molecular alteration, XIAP down-regulation in BLM cells led to a significant decrease in invasion of dermal skin equivalents.

Thus, XIAP down-regulation in BLM cells leads to reduced proliferation and migration, sensitizes melanoma cells towards necroptosis and, importantly, to altered invasion of metastatic melanoma cells. These results indicate that XIAP could serve as a pro-metastatic gene in skin melanoma and a therapeutic target for anti-cancer treatments.

P226

Dissecting heterogeneity in a melanoma short-term culture by single-cell RNAseq

T. Gerber[1], T. Kottek[2], H. Binder[3], E. Willscher[3], J. Utikal[4,5], L. Larribere[4], J. C. Simon[2], B. Treutlein[1] and M. Kunz[2] *[1]Max Planck Institute for Evolutionary Anthropology, Department of Evolutionary Genetics, 04103 Leipzig, Germany; [2]University of Leipzig, Department of Dermatology, Venereology and Allergology, 04103 Leipzig, Germany; [3]University of Leipzig, Department of Computer Science, 04107 Leipzig, Germany; [4]University Medical Center Mannheim, Department of Dermatology, Venereology and Allergology, 68167 Mannheim, Germany; [5]German Cancer Research Center, Skin Cancer Unit, 69120 Heidelberg, Germany*

Intratumoral heterogeneity is currently considered as the major reason for resistance and early recurrence after targeted treatment. Recent technological advances in single-cell genomics make it possible to analyse cellular heterogeneity within a tumor sample and, thus, may enable the finding of specific target molecules for tumor treatment.

Here, we used microfluidic single-cell RNA-seq to measure the transcriptome of 91 single cells obtained from a melanoma short-term culture. Principal component analysis followed by hierarchical clustering of the most variable genes revealed five major cell clusters that were mainly driven by cell cycle variation. Other functional categories that defined the different cell clusters included pigmentation, DNA repair, DNA damage response and cell adhesion. Analysis of the kinase expression pattern (cellular kinome) of the melanoma cell culture showed that CDK4 was consistently highly expressed in the vast majority of cells analysed. Similar findings were obtained for CDK2. CDK4 and CDK2 inhibitors were used for subsequent treatment of the melanoma cells and were more effective than classical MAPK inhibitors commonly used for melanoma treatment (e.g., BRAF, MEK1/2 and ERK1/2 inhibitors, respectively). Furthermore, a small cluster of cells showed overlapping gene expression signatures with those from neuronal crest stem cells and induced pluripotent stem (iPS) cells of fibroblast cultures, suggesting the presence of a small stem cell-like population among our single cells. Among the stem cell signature genes were ZEB2 (zinc finger E-box binding homeobox 2), FST (follistatin), PMP22 (peripheral myelin protein 22), and RHOB (ras homolog family member B), all of which play distinct roles in stem cell biology.

Taken together, we found genetic heterogeneity in a melanoma short-term culture which might reflect heterogeneity in primary melanomas or melanoma metastases. Clonal analysis identified CDK4 and CDK2 as promising targets for new treatment approaches in melanoma. Evidence was provided for the presence of a stem cell subpopulation in melanomas, which may provide a basis for more detailed studies in the future on the role of stem cells in this tumor.

P227

Interferon-alpha-based immunotherapy induces senescence in human cancer cells *in vivo*

T. Wieder[1], H. Braumüller[1], E. Brenner[1], C. Boss[1], K. Schilbach[2] and M. Röcken[1] *[1]University of Tuebingen, Dermatology, 72076 Tuebingen, Germany; [2]University of Tuebingen, General Pediatrics and Oncology/Hematology, 72076 Tuebingen, Germany*

Clinical studies using antibodies against so-called immune checkpoints, demonstrated the capacity of immunotherapy to control various types of metastatic cancer. Efficacy was first shown for melanomas. Several deathinducing mechanisms, e.g. apoptosis and cellular lysis, have been discussed to be responsible for the therapeutic effect. Yet, the clinical data suggest that immunotherapy may also activate non-toxic pathways leading to cancer control, namely cellular senescence, or permanent growth arrest. Cellular senescence is an important endogenous barrier against cancer development. In a recent study, we showed that cytokine-producing, tumor-specific T helper 1 cells are capable of arresting cancer growth by inducing senescence in endogeneous cancers. Furthermore, combined the T helper 1 cell cytokines interferon (IFN)-γ and tumor necrosis factor (TNF) drove different murine and human cancer cells into senescence *in vitro*.

Here, we investigated whether the therapeutically approved IFN-α likewise induces senescence in human rhabdomyosarcoma in a peritoneal tumor mouse model *in vivo*, and subsequently analyzed melanoma cells during IFN-α immunotherapy of patients suffering from life-threatening malignant ascites. We found that the combination of IFN-α and TNF, like IFN-γ and TNF, induced a stable state of senescence *in vitro*, as demonstrated by cell cycle analysis, permanent growth arrest, induction of senescence-associated-β-galactosidase (SA-β-Gal), and upregulation of the cell cycle inhibitor p16Ink4a. In the rhabdomyosarcoma mouse model, intraperitoneal application of IFN-α reduced the tumor load in the peritoneum more than ten-fold. In addition, the tumor cells isolated from IFN-α-treated mice were strongly growth inhibited, when cultured *ex vivo* in the absence of the cytokine. Similarly, intraperitoneal application of IFN-α into TNF-containing malignant ascites induced senescence in melanoma cells of two patients. Following IFN-α therapy, the melanoma cells expressed high levels of the senescence markers SA-β- Gal and p16Ink4a, whereas the proliferation marker Ki67 was strongly reduced. After three cycles of IFN-α, the melanoma cells became permanently growtharrested, also in the absence of further cytokine therapy, and were cleared from the peritoneum. Taken together, these data show in mice and in men that

cytokine-based immunotherapies may control the growth of malignant cancer cells *in vivo*. Thus, besides killing, cytokine-induced senescence is a non-toxic pathway that critically contributes to therapeutic cancer immune control also in humans.

P228

Dickkopf 3 (DKK3) deficiency delays the onset and progression of primary melanomas in HGF-CDK4 mice

N. Shridhar, T. Bald, C. Hüttner, M. Meyer and T. Tüting *Klinik und Poliklinik für Dermatologie und Allergologie, Department of Experimental Dermatology, 53127 Bonn, Germany*

Background: Dickkopf 3 (DKK3), belongs to the Dickkopf family of proteins and is involved in embryonal development. DKK3 is known to be a regulator of the wnt signaling pathway is expressed at low levels in many cancer cell lines including melanoma where it can act as a tumour suppressor. It is also expressed in tumour associated endothelial cells where it is thought to support angiogenesis. Recently it has been shown that DKK3 also has immunomodulatory functions. The role of DKK3 for the development and progression of melanoma is incompletely understood. In this study we aim to investigate the role of DKK3 in onset, progression and metastatic spread of melanoma in an experimental mouse model.

Methods: To address the role of DKK3 in melanoma pathogenesis we crossed HGFCDK4 (R24C) mice with DKK3-deficient mice. We compared onset, growth kinetics and metastasis of spontaneous as well as carcinogen DMBA induced cutaneous melanomas in a cohort of DKK3-deficient and -competent HGF-CDK4 (R24C) mice.

Results: The onset and progression of spontaneous melanomas was significantly delayed in DKK3-deficient HGF-CDK4 (R24C) mice. Accordingly we observed that survival of DKK3-deficient mice is significantly prolonged compared to DKK3-competent mice (Dkk3-deficient: 465 $\pm$ 68 days; Dkk3-competent: 268 $\pm$ 62). Phenotypically all melanomas in DKK3-deficient HGF-CDK4 (R24C) mice were homogenously pigmented and slow growing. DKK3-deficient HGF-CDK4 (R24C) mice lacked the fast growing, nodular melanomas which were observed in 43% of DKK3 competent HGF-CDK4 mice. Lymph node and lung Metastasis was similar in DKK3- deficient and competent HGF-CDK4 (R24C) mice. DMBA-induced melanomas also showed delayed growth in DKK3-deficient mice.

Conclusions: Taken together, our study shows that DKK3 acts as a tumour promoter in our genetically engineered HGF-CDK4 melanoma mouse model. Future investigations will have to elucidate the mechanism behind DKK3 delaying the growth and progression of melanoma, as a pre-requisite for therapeutic translation.

P229

Human melanoma cells show a broad range of responsiveness to type I interferons and susceptibility to oncolytic alphavirus infection

J. Ruotsalainen[1], A. Hinkkanen[2] and T. Tüting[1] [1]*Laboratory of Experimental Dermatology, University of Bonn, Department of Dermatology and Allergy, Bonn, Germany;* [2]*University of Eastern Finland, A.I. Virtanen Institute for Molecular Sciences, Kuopio, Finland*

Background: Oncolytic virotherapy is a new promising approach to treat malignant melanoma. Tumor cells are often permissive for viral infections due to their active metabolism and their decreased responsiveness to type I interferons (IFN). Recently, 70% human melanoma cell cultures were shown to be permissive for infection with oncolytic vesicular stomatitis virus due to a partially or severely compromised type I IFN response. The underlying mechanism for the type I IFN response defects in melanoma are poorly understood.

Aim: The aim of this work was to analyze the responsiveness of a collection of melanoma cell lines to type IFNs utilizing an oncolytic Semliki Forest Virus expressing EGFP (VA7-EGFP SFV) with the intention to elucidate the mechanisms underlying type I IFN response defects.

Materials and Methods: Human and mouse melanoma cells with a spectrum of phenotypes ranging from very melanocytic to poorly differentiated (as indicated by their expression of the MITF gene signature) were screened for their type I IFN responsiveness by treatment with varying concentrations of IFN- followed by infection with the oncolytic VA7- EGFP SFV. The infection kinetics were monitored with fluorescence and bright field microscopy over 72 h, after which the net result of cell proliferation was quantified using crystal violet staining. The melanoma cell lines were assigned a score for type I IFN responsiveness according to the lowest IFN concentration which still reduced viral oncolysis by at least 50% relative to the anti-proliferative effect of IFN alone. To further investigate the effect of the melanoma cell phenotype on viral oncolysis, melanosphere cultures were established for the human melanoma cell line MaMel15 and viral oncolysis was studied with VA7-EGFP SFV.

Results and Discussion: All melanoma cell lines were susceptible to high (MOI = 1) and medium (MOI = 0.01) doses of VA7-EGFP SFV without type I IFN pretreatment, resulting in significant cell death. When focal *in vivo* infection patterns were simulated *in vitro* by inoculating 10 infectious viral particles per well, half of the cell lines underwent efficient oncolysis. Following pretreatment with type I IFNs, 6 of the 12 human melanoma cell lines showed strongly reduced viral oncolysis, while in 4 cell lines viral oncolysis was only partially reduced and in two cell lines viral oncolysis was barely inhibited. Interestingly, the melanoma cell lines which were not protected from viral oncolysis by type I IFN pretreatment showed a very melanocytic (MITFhigh) phenotype, suggesting a potential link between the differentiation status and the responsiveness to type I IFNs. In support of this observation, the more differentiated mouse HCmel3 melanoma cells were also less responsive to the antiviral effects of type I IFNs than the less differentiated HCmel12 melanoma cells. In melanospheres of human MaMel15 cells, which consisted of a heterogeneous mixture of differentiated and undifferentiated cells, the rims were readily infected with VA7-EGFP SFV while the cores showed a delayed infection pattern. Taken together, our results provide preliminary evidence that a differentiated melanoma phenotype is less sensitive to the antiviral effects of type I IFNs and thus more susceptible for viral oncolysis. This warrants further investigations with the intention to guide the selection of melanoma patients suitable for oncolytic virotherapy and open up new possibilities for therapeutic combinatorial treatment approaches.

P230

The myelin protein PMP2 is regulated by SOX10 and drives melanoma cell invasion

S. A. Graf[1], S. Krebs[2], H. Blum[2], E. Hornig[1], C. Kammerbauer[1], A. Hamel[1], R. Besch[1] and C. Berking[1] [1]*Ludwig Maximilian University Munich, Department of Dermatology, 80337 Munich, Germany;* [2]*Ludwig Maximilian University Munich, Gene Center, 81377 Munich, Germany*

The transcription factor SOX10 plays a key role in the development of melanocytes and peripheral glia cells from neural crest precursors. Recently, SOX10 was found to be involved in melanoma initiation, proliferation, and survival. Furthermore, we identified SOX10 as a regulator of melanoma cell invasion by its target gene melanoma inhibitory activity (MIA).

To further investigate potential target genes of SOX10 we performed RNA sequencing with an ectopically SOX10 overexpressing metastatic melanoma cell line compared to control cells. A significant regulation was only found for a few genes and only peripheral myelin protein 2 (PMP2) was upregulated by SOX10 in three different melanoma cell lines. The fatty acid binding protein PMP2 together with myelin basic protein (P1) and myelin protein zero (P0) are the most abundant myelin proteins in the peripheral nervous system. PMP2 is predominantly expressed in myelinated Schwann cells where it transports fatty acids to membranes and thus plays a role in lipid homeostasis.

We found PMP2 to be downregulated by SOX10 inhibition and detected mRNA expression in melanocytes and melanoma cell lines. However, protein expression was not present in fibroblasts ad melanocytes and was restricted to a few melanoma cell lines.

Inhibition of PMP2 in PMP2-positive cell lines reduced cell number, morphology, and cell viability about 3 days after siRNA transfection and increased p21 levels. However cell viability was not increased upon PMP2 overexpression. Interestingly, stable PMP2 expression in a PMP2 cell line of radial growth phase, significantly increased invasion compared to a PMP2 mutant and control cells.

Direct binding of SOX10 to the PMP2 promoter was shown by chromatin immunoprecipitation and electrophoretic shift assays.

SOX10 is also essential for the development of Schwann cells. Furthermore, SOX10 was found to regulate myelin proteins in these cells. Previous studies indicate that SOX10 together with the transcription factor Egr2 regulates PMP2 expression and both SOX10 and Egr2 are expressed in undifferentiated and mature myelinating Schwann cells. We could also see a co-regulation of PMP2 expression by SOX10 and Egr2 in melanoma cells. Furthermore, SOX10 inhibition is able to reduce other proteins with specific functions in developing Schwann cell and melanoma cells. Therefore SOX10 might be a specific regulator of proteins which functions are essential for Schwann cell and melanocyte development but which also function in melanoma.

Considering targeted therapy, pleiotropic effects on the expression of a multitude of genes resulting in unacceptable toxicity by inhibition of SOX10 are most likely. Thus inhibiting SOX10 target genes, such as MIA and PMP2, which are involved in melanoma cell invasion might be a successful strategy to prevent metastasis.

P231

Hyperactive NRAS downstream signaling induces specific transcriptome and phosphoproteome changes – identification of new therapeutic targets in NRAS mutant melanoma

I. Vujic[1,2], M. Vujic[1,2], M. Sanlorenzo[2,3], R. Esteve-Puig[2], C. Posch[1,2], K. Lin[2], K. Lai[2], K. E. Wu[2], D. Lee[2], W. Ho[2], K. Rappersberger[1] and S. Ortiz-Urda[2] [1]*Rudolfstiftung Hospital, Dermatology, 1030 Vienna, Austria;* [2]*University of California, San Francisco, Dermatology, San Francisco, USA;* [3]*University of Turin, department of medical sciences, Section of Dermatology, Turin, Italy*

Activating NRAS mutations in melanoma are common and new immunomodulatory therapies have improved outcomes in some patients with this aggressive cancer. Still, the majority of tumors eventually progress and there are no approved small molecule therapies for this deadly cancer. Such NRAS mutations lead to increased downstream signaling and thus to possible changes in the transcriptome and phospho-proteome in melanocytes and tumors. The knowledge of these changes can (i) explain why some melanocytic naevi have NRAS mutations but never progress to cancer, and (ii) lead to the development of new therapies and to a better understanding of NRAS mutant melanoma biology.

Here, we introduce NRAS mutant plasmids in a pool of primary human melanocytes. We perform deep RNAseq analyses and observe transciptome changes in melanocytes induced by NRAS downstream pathway hyperactivation. Next we compare the results RNASeq data of two NRAS mutant melanoma cell lines with hyperactive NRAS signaling. We integrate this data with RNASeq data of 86 NRAS mutant melanoma patients and define a list of coding and noncoding genes which are differentially expressed in NRAS mutant melanoma tumors and NRAS mutant melanocytes compared to normal melanocytes. Additionally we use stable isotope labeling by amino acids in cell culture (SILAC) in NRAS mutant and non-mutant melanocytes and describe how the hyperactvation of the NRAS downstream pathways changes the phospho-proteome. Finally we intergrate the transcriptome and phosphoproteome data and identify new therapeutic targets in NRAS mutant melanoma.

P232 withdrawn

P233 (O03/01)

TLR4 signaling is crucial for the α-MSH-mediated inhibition of MDSC expansion and tumor progression

N. Sucker[1], C. Weishaupt[1], T. Vogl[2], J. Roth[2], T. A. Luger[1] and K. Loser[1] [1]*University of Muenster, Department of Dermatology, 48149 Muenster, Germany;* [2]*University of Muenster, Institute of Immunology, 48149 Muenster, Germany*

In a two-stage chemocarcinogenesis model we have previously shown that the anti-inflammatory neuropeptide alpha-melanocyte-stimulating hormone (α-MSH) protected mice from developing skin tumors. This effect was mediated by the induction of tumor-specific CD8+ cytotoxic T lymphocytes (CTL). Since the expansion and function of anti-tumoral effector cells can be controlled by myeloid-derived suppressor cells (MDSC), we quantified MDSC and could demonstrate that α-MSH, via binding to the melanocortin-1 receptor (Mc-1r), prevented the expansion of MDSC in DMBA/TPA-treated mice as well as in patients with basal cell (BCC) or squamous cell carcinomas (SCC) indicating that α-MSH up-regulated MHC class Imediated anti-tumoral immunity by inhibiting MDSC. To investigate the molecular mechanism underlying the α-MSH-mediated inhibition of MDSC expansion in more detail we characterized NF-κB signaling and analyzed the TLR4 pathway in mice with epithelial tumors before and after treatment with α-MSH since it has been shown that ligation of TLR4 by the damage-associated molecular pattern (DAMP) proteins S100A8 and S100A9 and the subsequent activation of NF-κB signaling is essential for MDCS generation. Interestingly, in tumors from α-MSH treated mice we detected significantly reduced levels of S100A8 and S100A9 compared to PBS-treated controls pointing to the down-regulation of TLR4 signaling by α-MSH. In support of this, the expression of CD14 and IRAK-1, well known signaling proteins downstream of TLR4, was markedly reduced after α-MSH versus PBS treatment. Hence, or data suggested that α-MSH, by inhibiting the expression of the DAMP proteins S100A8 and S100A9, down-regulated TLR4 signaling finally resulting in the reduction of MDSC expansion. To scrutinize this hypothesis we performed a two-stage skin carcinogenesis in mice deficient for the DAMP proteins S100A8 and S100A9. Strikingly, α-MSH treatment did neither reduce tumor development nor prevent the expansion of MDSC in mice deficient for S100A8 and A9, thus clearly indicating that the α-MSH-mediated suppression of MDSC was dependent on S100A8/A9 and TLR4 signaling. Ligation of TLR4 by S100A8 and S100A9 proteins results in the activation of NF-KB. Usually in its inactive form, NF-κB can be found in the cytoplasm and is bound to IκBα. Upon activation via both, the canonical and non-canonical pathway, IκBα is phosphorylated and degraded resulting in the release of NF-KB from the complex and enabling its translocation into the nucleus leading to the induction of target gene transcription. To assess the role of α-MSH on NF-KB activation in tumors from mice treated with the neuropeptide, we quantified total NF-kB and IkBa as well as its active forms using the Luminex technology. Interestingly, tumors of α- MSH-treated mice showed a significantly reduced expression of the phosphorylated, and therefore active, forms of NF-κB and IκBα compared to PBS-treated controls. Together, our data demonstrate that in mice and humans with epithelial skin tumors, α-MSH, by binding to Mc-1r down-regulates the DAMP proteins S100A8 and A9 resulting in the inhibition of TLR4 signaling and NF-κB activation, finally leading to the suppression of MDSC expansion and the up-regulation of MHC class I-restricted antitumoral immunity.

P234

CXCL5 alters metastatic patterns of malignant melanoma

A. Forsthuber[1], K. Lipp[1], A. Soler-Cardona[1,2] and R. Loewe[1,2] [1]*Medical University of Vienna, Skin and Endothelium Research Division, Department of Dermatology, 1090 Vienna, Austria;* [2]*Medical University of Vienna, Division for General Dermatology and Dermatooncology, Department of Dermatology, 1090 Vienna, Austria*

Previous analysis of human and mouse melanoma chemokine profiles showed that high expression of CXCL5 is in accordance with a worse disease progression in terms of lymph node metastasis. To investigate the role of CXCL5, an immune competent melanoma C57BL/6 mouse model, using CXCL5/LIX overexpressing B16F1 cells, was established. CXCL5 expressing melanoma strongly recruited neutrophils to the primary tumor and showed higher frequencies of lymph node metastasis than the wt control tumors. Additionally, metastasis of CXCL5 expressing tumors was restricted to a lymphogenic route, whereas the wt control tumors metastasized via lymphatic vessels as well as blood vessels.
Chemokine profiling of CXCL5 overexpressing tumors versus control shows that changing the expression of one single chemokine does not affect the expression pattern of other well-known pro tumorigenic chemokines. This gives CXCL5 and its recruited neutrophils more importance being the active key players in melanoma lymph node metastasis. *In vivo* experiments using a neutrophil depletion antibody and a CXCL5 neutralizing antibody will unravel the specific effects of neutrophils and CXCL5 separately on disease progression. Additionally, samples from human melanoma xenografted in SCID mice as well as melanoma patient samples will be analysed for the presence of CXCL5 and correlated to disease outcome.

P235

Analysis of the transcription factor JunB in cytokine-induced cancer cell senescence

N. Simon, R. Seeger, T. Wieder, J. Bauer, H. Braumüller and M. Röcken *University Medical Center Tuebingen, Dermatology, 72076 Tuebingen, Germany*

Tumor immunotherapy has recently become highly relevant for treatment of melanoma and other cancers. Efficient immunotherapy of cancer either results to cancer cell apoptosis or killing, alternatively it may induce a stable growth arrest. This stable growth arrest, also known as senescence, can be induced by the Th1 cytokines interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα) *in vitro* and *in vivo*. Previous data showed an upregulation of the tumor suppressor gene p16INK4a, but the exact molecular pathway by which cellular senescence is induced is unknown. A possible candidate is JunB. This transcription factor is known as a target of the TNF signaling pathway, but an interaction between JunB and the p16INK4a promoter has not been described, especially not in the context of cytokineinduced senescence. Furthermore, it is unclear whether JunB can bind directly to the p16INK4a promoter or if binding requires the association in a protein complex, for example AP-1.
In order to investigate this signaling pathway in detail, we analyzed murine cell lines isolated from the pancreas of RIP-Tag2 mice. In this model, the SV40 large T antigen 2 (Tag2) is expressed under the rat insulin promoter (RIP). Similar to the E6/E7 genes of HPV-induced bowen carcinoma, or PyV-genes in Merkel cell carcinoma, Tag causes an inhibition of p53 and Rb1 exclusively in the Langerhans islets cells leading to a multistage carcinogenesis.
Our data show a translocation of JunB from the cytoplasma into the nucleus after combined cytokine treatment. In addition, we found an upregulation of JunB protein, starting 4 h after cytokine treatment and increasing for 8 h in RIP-Tag2 mice.
Similary, IFNγ and TNFα reduced JunB in polyoma middle T (PyT)-driven cancers with similar dynamics. First qPCR analyses suggest induction of JunB mRNA as early as 2 h after stimulation and persisting for at least 8 h.
Together, our findings suggest a central role of JunB in the early phase of cytokineinduced senescence. JunB most likely interacts as part of a protein complex with the promoter of p16INK4a. TNF1 receptor knock-out cells will unravel whether JunB is activated exclusively by the TNF signaling or if further signals are needed in addition.

P236

Extrinsic or intrinsic apoptosis by curcumin and light: still a mystery

N. Zöller, V. Laubach, M. Butting, M. Hofmann, R. Kaufmann, A. Bernd and S. Kippenberger *University Hospital, Department of Dermatology, Venereology and Allergology, 60590 Frankfurt, Germany*

Curcumin, a dietary pigment from the plant Curcuma longa, is well known for its ability to inhibit cell proliferation and induce apoptosis in different cell lines. In previous studies we showed that low curcumin concentrations (0.2–1 μg/ml) and subsequent irradiation with UVA or visible light induced anti-proliferative and proapoptotic effects in different cell lines. There is still debate whether curcumin induces apoptosis via the extrinsic or the intrinsic pathway. In two cell lines we investigated whether the death receptors CD95, TRAIL-1, TNF-receptors 1 and 2 were involved in apoptosis induced by light and curcumin.
HaCaT and A549 cells were incubated with 0.25–0.5 μg/ml curcumin followed by irradiation with 1 J/cm^2 UVA. Death receptor specific apoptosis inducers as well as death receptor inhibitors were applied after the curcumin/light combination treatment. After 24 h apoptosis induction was monitored by Western blot analysis and quantitative determination of cytoplasmic histone-associated-DNA-fragments.
We evaluated our test system and the applicable agonists' and antagonists' concentrations by showing that the FAS agonist, CH11, induced apoptosis could be completely inhibited by adding the FAS antagonist ZB4. The death receptor ligands TNF-α and TRAIL and their specific inhibitors were likewise evaluated.
We found that addition of the FAS antagonist, ZB4, and also antagonists against TNF-receptor 1/2 and TRAIL does not influence the apoptosis induced by curcumin/light treated cultures in both cell lines. The results indicate a signalling independent from classical death receptors.

P237

mTOR mediated insulin resistance as a potential pathomechanism in malignant melanoma

V. Lang, F. Toiussant, S. Diehl, M. Meissner, R. Kaufmann and C. Buerger *University Hospital of the Goethe University, Department of Dermatology, Frankfurt, Germany*

Malignant melanoma is one of the most aggressive cancers and despite a growing number of promising therapeutic approaches, the prognosis remains poor for most patients. There is evidence that the risk for several cancer types like pancreatic, hepatic, colorectal and breast cancer is increased in diabetic patients and that molecular insulin resistance may represent a pathomechanisms in carcinogenesis.
In malignant melanoma this correlation is still unclear. Nevertheless first indications of a potential association between obesity and insulin resistance as an independent risk factor have been pointed out. Thus we investigated, whether molecular insulin resistance contributes to carcinogenic alterations in different melanoma cells.
We could previously show that under norminsulinemic conditions melanoma cell lines show constitutive mTOR and MAPK activity that cannot be further enhanced by insulin treatment, while Akt is sensitive to insulin stimulation. However, under conditions of chronic hyperinsulinemia, Akt activity cannot be induced by short term insulin treatment, which is characteristic of molecular insulin resistance. In contrast healthy melanocytes still respond with Akt activation under these conditions. Blocking mTOR or MAPK activity with either rapamycin or U0126 restores insulin sensitivity suggesting that oncogenic hyperactivation of these kinases contributes to molecular insulin resistance.
We then asked what physiological consequences are mediated by this molecular insulin resistance. Measuring cell proliferation we found that insulin only has a small supporting effect on cell growth. The genetic activation of the MAPK pathway in melanoma may be a reason for the low influence of additional external growth signals. This is supported by the finding that blockade of MAPK signaling using U0126 strongly suppressed cell proliferation. In addition we found that chronic hyperinsulinemia upregulates certain adhesion factors such as $\alpha V\beta 3$ integrin, which could point towards a role of deregulated insulin signaling in cell migration and metastasis. However in scratch assays chronic insulin exposure did not have an effect on cellular migration.
Although the cellular consequences of molecular insulin resistance in melanoma remain to be determined, we found that IRS-1 is hyper-phosphorylated at serine 636/9 in melanoma tissue and correlates with tumor progression. Phosphorylation of IRS-1 at this site is mediated by mTOR signaling, which destabilizes IRS-1 and therefore is indicative for insulin resistance.
In summary we present evidence that hyper-activation of mTOR signaling contributes to insulin resistance in malignant melanoma which in turn potentially contributes to tumor development. Thus, exploring the use of mTOR inhibitors for the treatment of melanoma is not only reasonable because of their anti-proliferative properties, but also could be therapeutically favorable by normalizing insulin signaling.

P238 (O05/03)

Tumor cell intrinsic TLR4 signaling promotes growth and metastasis in melanoma

M. Rogava[1], T. Bald[1], N. Shridhar[1], D. van den Boorn-Konijnenberg[2], J. Schmid- Burgk[3], V. Hornung[3], M. Hölzel[2] and T. Tüting[1] *[1]University Hospital, Department of Dermatology, 53127 Bonn, Germany; [2]University Hospital Bonn, Institute for Clinical Chemistry and Clinical Pharmacology, 53127 Bonn, Germany; [3]University Hospital Bonn, Institute of Molecular Medicine, 53127 Bonn, Germany*

Metastatic melanoma is a leading cause of death in skin cancer worldwide. We found that Toll-like receptor 4 (TLR4) signaling in the tumor microenvironment is important for melanoma cell metastasis in our primary and transplantable HGFCDK4 mouse melanoma. Accumulating evidence in the literature shows that TLRs are important on tumor cells too. We hypothesized that tumor cell intrinsic TLR4 signaling also contributes to melanoma cell survival and metastasis. To address this hypothesis, we utilized a HGF-CDK4 melanoma cell line established in our laboratory. We generated TLR4 deficient melanoma cell variants using the CRISPR/CAS9 genome editing technology. TLR4 deficient clones were validated by Next Generation Sequencing and in functional assays. As a next step, TLR4 deficient tumor cells were transplanted in immunocompetent syngeneic C57BL/6 mice and monitored for tumor growth and metastasis. Here we show that melanoma cells functionally express TLR4. Activation of TLR4 signaling in melanoma cells increases proliferation and migration *in vitro*. TLR4 deficient cells demonstrate delayed growth kinetics upon transplantation in immunocompetent mice *in vivo*. Furthermore, TLR4 deficient melanoma cells also show decreased numbers of metastases in the lungs. Genetic reconstitution of TLR4 in melanoma cells reversed the observed impairment in tumor growth and metastasis. Taken together, our results provide experimental evidence that TLR4 signaling has melanoma cell-intrinsic function and promotes survival and metastasis. This further supports strategies to inhibit TLR4 signaling as an adjuvant treatment option for melanoma patients with a high risk for metastatic dissemination.

P239 (O06/05)

RB1 is the crucial target of the Merkel cell polyomavirus large T antigen in Merkel cell carcinoma cells

D. Schrama, S. Hesbacher, L. Pfitzer and R. Houben *University Hospital Wuerzburg, Dermatology, Wuerzburg, Germany*

The pocket protein (PP) family consists of the three members RB1, p107 and p130 all possessing tumor suppressive properties. Indeed, the PPs jointly control the G1/S transition mainly by inhibiting E2F transcription factors. Notably, several viral oncoproteins are capable of binding and inhibiting PPs. Thus, we analyzed the interaction of Large T antigen (LT) of the Merkel cell polyomavirus (MCPyV) with the PPs. MCPyV is established as etiological vector for Merkel cell carcinoma (MCC) with LT expression in MCC cells required for their proliferaton. Co-IP experiments indicate that MCPyV-LT potently binds only to RB1. Moreover, MCPyVLT knockdown induced growth arrest in MCC cells can be rescued by knockdown of RB1, but not by p107 or p130 knockdown. Accordingly, cell cycle arrest and E2F target gene repression mediated by the single PPs can only in the case of RB1 be significantly reverted with MCPyV-LT. Moreover, data from an MCC patient demonstrate that by loss of RB1 the MCC cells become MCPyV-LT independent. Thus, our results suggest that RB1 is the dominant tumor suppressor PP in MCC, and that inactivation of RB1 by MCPyV-LT is largely sufficient for its growth supporting function in established MCPyV-positive MCC cells.

P240

Cytokine-induced senescence of cancer cells involves argonaute protein 2

M. Rentschler, Y. Chen, H. Braumüller, J. Pahl, E. Brenner, S. Weidemann, T. Wieder and M. Röcken *University Medical Center Tuebingen, Department of Dermatology, 72076 Tuebingen, Germany*

In normal cells, overexpression of oncogenic HRAS (HRASG12V) leads to permanent cell cycle arrest, a phenomenon Serrano et al. named oncogene-induced senescence. As oncogene-induced senescence occurs in premalignant lesions *in vivo*, it is now considered to be an intrinsic tumor control mechanism. In this line, we found that tumor control can also be induced by exogenous, cytokine-dependent signals. We showed that adaptive immunity and the combined action of the T helper 1 cell (Th1) cytokines interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) drive cancer cells into senescence. Cytokine-induced senescence (CIS) mainly depends on activation of the p16INK4a/Rb signaling pathway, as well as on the consecutive inactivation of the E2F family of transcription factors. However, the exact regulatory mechanism remained enigmatic. Since argonaute protein 2 (Ago2), which is part of the RNA-induced silencing complex (RISC), is known to induce heterochromatin foci in oncogene- or doxorubicin-induced senescence and to suppress E2F target genes, we analyzed its role in CIS. Treatment of different human cancer cell lines with IFN-γ and TNF-α permanently stopped cellular proliferation in the absence of cell death, and increased the activity of senescence-associated β-galactosidase. We then tested the expression and localization of Ago2 protein after cytokine challenge using immunofluorescence staining. As expected, all cancer cells showed Ago2 expression independent of the culture conditions. Yet, following treatment with the cytokine cocktail, Ago2 translocated from the cytoplasm into the nucleus in Ki67-negative, non-proliferating cancer cells. Nuclear translocation of Ago2 occurred after 24–48 h of treatment, and can thus be considered as an early event in the induction phase of CIS. Moreover, knockdown of Ago2 by an siRNAmediated approach released the cancer cells from cytokine-induced growth arrest. As Ago2 translocated into the nucleus of non-proliferating cells, probably as a corepressor of the E2F/Rb complex, Ago2 significantly contributes to senescence induction in human cancers. Thus, CIS is an important tumor suppressor mechanism that permanently stops the proliferation of human cancer cells, which is partly regulated by Ago2.

P241

Epigenetic and transcriptional regulation of Brn3a in melanoma

E. Hornig, M. Heppt, S. A. Graf, C. Kammerbauer, T. Hohenauer and C. Berking *Ludwig-Maximilian University, Department of Dermatology and Allergology, 80337 Munich, Germany*

Brn3a is a transcription factor of the Pit-Oct-Unc (POU) domain that is involved in neural crest development. Strong expression of Brn3a was previously shown in a panel of melanoma cells and patient-derived tumor samples but not in primary cells of the skin. However, the mechanisms underlying aberrant expression of Brn3a remain elusive. Here, we investigate the regulation of Brn3a in the melanocytic lineage. We analysed expression of BRN3a after inhibition of histonedeacetylases (HDACs) in different cell types with pharmacologic HDAC inhibition and specific gene silencing with siRNA. Treatment with the pan HDAC inhibitors trichostatin A and sodium butyrate led to rapid and strong up-regulation of Brn3a in melanocytes and melanoma cells, but not in fibroblasts. Other neural crest genes like Brn2 or Sox9 were left unaltered, suggesting that regulation through acetylation is specific for Brn3a. Selective inhibition of the HDACs 1, 2, 3, and 11 with Mocetinostat went along with increased expression of Brn3a. To further identify single HDACs that contribute to Brn3a regulation, we analysed expression of different HDACs in melanoma cell lines and correlated them to the respective Brn3a expression levels. The HDACs 2, 4, 9 and 11 showed an inverse correlation to the Brn3a expression. A specific knockdown of HDAC 2 and HDAC 11 with siRNA resulted in an upregulation of Brn3a levels in melanocytes, implying that both are involved in its epigenetic regulation. In melanocytes, we hypothesize that the gene locus for Brn3a is silenced through either epigenetic mechanisms or transcriptional regulation. As we could not detect any induction of Brn3a in fibroblasts, we conclude that there is a melanocyte-specific transcription factor driving expression of Brn3a in addition to acetylation. Therefore, we truncated luciferase-based promoter constructs of the human Brn3a promoter and identified a putatively active promoter area spanning 200 Bp proximal to the Brn3a gene. Taken together, the regulation of Brn3a in melanoma and melanocytes is a complex process driven by acetylation and specific transcription factors. The strong and rapid induction after pharmacologic and specific inhibition of HDAC 2 and 11 underlines that epigenetics may substantially confer malignant transformation of melanocytes.

P242

Von Willebrand factor fibers in tumor vasculature mediate tumor progression and inflammation in malignant melanoma

J. R. Robador[1], A. T. Bauer[1], L. Görtz[1], J. Suckau[2], A. Desch[1], V. Umansky[3,4] and S. W. Schneider[1]
[1]Heidelberg University, Medical Faculty Mannheim, Experimental Dermatology, 68167 Mannheim, Germany; [2]University Hospital of Erlangen, Plastic and Hand Surgery, 91054 Erlangen, Germany; [3]German Cancer Research Center (DKFZ), Heidelberg, Skin Cancer Unit, 69121 Heidelberg, Germany; [4]Heidelberg University, Medical Faculty Mannheim, Dermatology, Venereology and Allergology, 68167 Mannheim, Germany

It is well known that patients with malignant melanoma hold a high risk for venous thromboembolism associated with a worse prognosis due to a high incidence of metastasis. Prior to form metastasis in distant organs, a tumor cell needs to interact with endothelial cells (ECs) of the vessel wall and extravasate. Our previous *in vitro* studies show that melanoma cells interact with ECs via different mechanisms. First, using an indirect pathway mediated by tissue factor of tumor cells promoting thrombin generation. Second, we could identify tumor cell-secreted VEGF-A as key molecule for a direct interaction of tumor cells with ECs. Both pathways promote EC activation and the release of von Willebrand factor (VWF) forming VWF fibers on the luminal surface of the endothelium. Furthermore, we could demonstrate the existence of intraluminal VWF fibers in tumor blood vessels of mice and patients promoted by a strong activation of ECs and inhibition of the protease ADAMTS13 (a disintegrinlike and metallopreteinase with thrombospondin type I repeats 13), which degrades and inactivates VWF fibers. In the presented study, we evaluated the impact of EC activation and VWF fiber formation on tumor progression using different mouse models (intradermal and intravenous inoculation of melanoma cells).

Immunofluorescence analyses revealed that VWF fibers occur in blood vessels of primary tumors, lung metastases and in tumor-primed tissue of distant organs without detectable metastasis. Using ADAMTS13 deficient mice, characterized by a prolonged persistence of VWF fibers, we observed a positive correlation between the presence of VWF networks, increased thrombus formation, angiogenesis, tumor growth and lung metastasis. What is even more, we detected intraluminal neutrophils releasing extracellular DNA which strongly interacts with VWF fibers in the tumor vasculature. Modulation of EC activation using the anticoagulant low-molecularweight heparin (LMWH) Tinzaparin blocked VWF fiber formation and platelet aggregation. Interestingly, a strong increase of neutrophils within the lung tissue was associated with strongly reduced lung metastasis upon Tinzaparin treatment.

In conclusion, our results provide new aspects of VWF function and processing and envision a sound molecular explanation of tumor-associated thrombosis and inflammation. Furthermore, inhibition of EC activation or microthrombi formation may provide new therapeutical strategies for the treatment of malignancy using clinically approved LMWHs, such as Tinzaparin, an anticoagulant recommended in cancerassociated thromboembolism.

P243

Expression of CD164 on malignant T cells in Sézary syndrome

D. Ignatova[1,2], Y. Chang[1], E. Contassot[1], T. Mehra[3], V. Mitev[2], R. Dummer[1], L. E. French[1], W. Hoetzenecker[1], A. Cozzio[1] and E. Guenova[1] *[1]University Hospital Zurich, Department of Dermatology, 8091 Zurich, Switzerland; [2]Medical University, Sofia, 3Department of Medical Chemistry and Biochemistry, Sofia, Bulgaria; [3]University Hospital Zurich, Medical Directorate, 8091 Zurich, Switzerland*

Sézary syndrome is a primary cutaneous T cell lymphoma characterized by pruritic erythroderma, peripheral lymphadenopathy, and the presence of malignant T cells in the blood. Unequivocal detection of malignant cells in Sézary Syndrome patients is of important diagnostic, prognostic and therapeutic value and is essential for disease monitoring under treatment. However, a single Sézary syndrome specific cell surface marker has not yet been identified. In a cohort of Sézary syndrome patients, CD164 expression on total CD4+ lymphocytes was significantly upregulated compared to healthy controls. CD164 expression was in most cases limited to CD4+CD26− malignant T lymphocytes, unequivocally flow-cytometrically identified by the expression of a specific Vβ clone for each patient. Increased expression of CD164 may be a promising diagnostic parameter and a potential target for a CD164- linked therapeutic approach in Sézary syndrome.

P244 (O02/04)

Impaired UV – induced angiogenic response in the skin of TNF-deficient mice

M. Sonneck, T. Bald, T. Tüting and E. Gaffal *Experimental Dermatology, Bonn, Germany*

Background: Exposure to ultraviolet radiation (UV) induces a neutrophil rich inflammatory response in the skin. Previously, we demonstrated that repetitive UV irradiation of primary and transplanted Hgf-Cdk4 melanomas promotes the expansion of tumor cells along abluminal blood vessel surfaces and increases the number of lung metastases dependent on a neutrophilic inflammation. Since host-derived TNF fosters the interaction of melanoma and endothelial cells *in vitro*, the central aim of this work is to investigate the impact of TNF for an UV-induced skin inflammatory response and melanoma progression *in vivo*.

Methods: Thus, we studied skin inflammatory responses in TNF-competent and – deficient C57BL/6 mice after two sunburning doses (4.5 kJ/m^2) of UVB compared to TPA-treated and untreated controls. We characterized immune cell infiltration by flow cytometry and the angiogenic response by histology.

Results: Exposure to UV irradiation, or TPA treatment, induced a similar increase of immune cells, predominantly of the myeloid lineage, in the skin and blood of TNF-competent and -deficient C57BL/6 mice. The reactive proliferative response of keratinocytes was also comparable while the thickness of the dermis of TNF-deficient mice was reduced after UV irradiation or TPA-treatment. Interestingly, macroscopic analyses of back skin flaps showed a reduced angiogenic response, suggesting reduced vessel dilatation and vessel leakage, in TNF-deficient compared to TNFcompetent mice. We are currently investigating the differences in the angiogenic response in TNF-competent and -deficient C57BL/6 mice using the Miles Assay. Furthermore, we will investigate the impact of TNF on metastatic progression of transplanted Hgf-Cdk4 melanoma cells.

Conclusions: Taken together, the results show that TNF seems to be required for the UV-induced angiogenic response without critically impairing neutrophilic inflammatory cell infiltrates. Given the importance of tumor cell – endothelial cell interactions for metastatic progression and therapy resistance, we believe that unraveling the role of TNF during these processes might help to develop new treatment approaches for melanoma.

P245

Preclinical evaluation of the compound 4SC-202, a combined LSD1- and HDACinhibitor, for the treatment of cutaneous T cell lymphoma

M. Wobser[1], A. Weber[1], A. Glunz[1], H. Kohlhof[2], M. Goebeler[1], D. Schrama[1] and R. Houben[1]
[1]University Hospital Wuerzburg, Dermatology, 97080 Wuerzburg, Germany; [2]4SC AG, 82152 Planegg, Germany

Targeting of epigenetic mechanisms such as histone methylation and acetylation has been proven to be effective in several malignancies. In this regard, 4SC-202 is a novel compound, which has been demonstrated to dually inhibit class 1 histone deacetylases (HDAC) as well as the lysine (K)-specific demethylase 1A (LSD1), a monoaminoxidase capable of demethylating mono- and di-methylated lysines. 4SC-202 is currently investigated in a phase 1 trial (TOPAS) in patients with advanced hematologic malignancies.

Since HDAC inhibitors have demonstrated clinical efficacy in all stages of cutaneous T cell lymphomas (CTCL), we wanted to assess the impact of combined HDAC and demethylase inhibition on CTCL. Therefore, we first analyzed LSD1 expression in tissue of CTCL by immunohistochemistry. To this end, LSD1 exhibited a stage-dependent incremental expression level in mycosis fungoides with highest expression in tumor stage. Consequently, we assessed the impact of 4SC-202 on the growth of CTCL cell lines by using MTS assays. These analyses demonstrated that all six different CTCL cell lines tested were strongly inhibited by the compound, irrespective of the level of LSD1 or HDAC expression as determined by qPCR. In contrast, fibroblasts or peripheral blood lymphocytes were largely resistant towards 4SC-202. Subsequent cell cycle analyses revealed that growth inhibition of CTCL lines is due to an arrest in G2/M followed by induction of massive cell death. Interestingly, when comparing 4SC-202 with the well-studied HDAC class I inhibitor FK228 at concentrations resulting in similar levels of cell death, 4SC-202 had only minor effects on the global histone acetylation pattern with respect to functionally relevant sites (H3K9ac), however, induced an increase of dimethyl H3K4 levels. Currently ongoing experiments will uncover the underlying mechanisms of efficient growth inhibition of 4SC-202 on CTCL cell lines.

P246

Regulation of Weibel Palade body content and endothelial cell function by melanoma derived exosomes

T. Downar[1], C. Gorzelanny[1], J. Hummel[1], T. Worst[2], P. Erben[2] and S. W. Schneider[1] *[1]Medical Faculty Mannheim, Heidelberg University, Experimental Dermatology, 68167 Mannheim, Germany; [2]Medical Faculty Mannheim, Heidelberg University, Department of Urology, 68167 Mannheim, Germany*

Introduction: Exosomes, defined by a size between 30 nm and 100 nm, are small extracellular vesicles secreted from every kind of cell. Melanoma-derived exosomes are known to drive distant metastasis formation into lung and brain tissues. However, the underlying pathophysiological mechanisms are yet not well understood. In the present work we aim to clarify whether exosomes are able to modulate the composition of endothelial Weibel Palade bodies, large intracellular vesicles loaded with factors regulating inflammation, vascular permeability and coagulation.

Material and Methods: Exosomes were purified from human melanoma cell supernatants (BLM, SK-Mel 30, Ret, B16/F10) by several centrifugation, filtration and ultracentrifugation steps. Effect of exosomes on human umbilical vein endothelial cells (HUVECs) were measured by qPCR, ELISA, electric cell-substrate impedance sensing and fluorescence microscopy.

Results: Exosomes isolated from cell supernatants were characterized by electron microscopy and by nanoparticle tracking analysis (NTA) documenting an exosome count of $2.7 \times 108 \pm 1.4 \times 107$particle/ml and a mean size of 117 ± 4.9 nm. HUVECs were treated with various concentrations of exosomes ranging from 14 to 240 exosomes per single endothelial cell. Fluorescence microscopic investigation of the exosome uptake into HUVECs suggests a juxtacrine signalling pathway that triggers exosome-mediated regulation of endothelial cell function. Gene expression profiling of 21 Weibel Palade body-related proteins by qPCR indicated a strong dose-dependent regulation of several genes. Low concentrations of exosomes (14–55 exosomes/HUVEC) attenuated the expression of pro-inflammatory genes such as interleukin-8 or eotaxin-3. This down-regulatory effect of exosomes on the interleukin-8 expression was confirmed on the protein level by ELISA. In contrast, high concentrations of exosomes (240 exosomes/HUVEC) were found to mediate a significant upregulation of genes, associated with vascular permeability such as angiopoetin-2 or VEGF-A. The expression of genes related to coagulation such as tissue factor or thrombomodulin were not significantly affected. Electric cellsubstrate impedance sensing indicated a reduction of the trans-endothelial electrical resistance upon high dose exosome treatment. These results further substantiated the regulatory impact of exosomes on the vascular permeability.

Conclusion: Our work indicated that exosomes control the regulation of several endothelial genes which might also alter the composition of Weibel Palade bodies and thus endothelial cell functionality. While the anti-inflammatory effect of exosomes might hinder the recognition of circulating melanoma cells through the immune system, an exosome mediated attenuation of the vascular barrier could promote tumor cell extravasation prior to metastasis formation.

P247

Senescent fibroblasts enhance cutaneous squamous cell carcinoma progression through secretion of Chemerin and activation of MAPK pathway

V. Farsam[1], A. Basu[1], M. V. Gatzka[1], M. Wlaschek[1], M. A. Mulaw[2], T. Lucas[3], S. Kochanek[3] and K. Scharffetter-Kochanek[1] *[1]University of Ulm, Department of Dermatology and Allergic Diseases, Ulm, Germany; [2]University of Ulm, Institute of Experimental Cancer Research, Ulm, Germany; [3]University of Ulm, Department of Gene Therapy, Ulm, Germany*

Cutaneous squamous cell carcinoma (cSCC) is the second most common type of skin cancer worldwide with an increased propensity for local recurrence and metastasis. The incidence of cSCC rises dramatically with age which is proposed to be not only due to accumulation of mutations but also age-related alterations in tissue stroma. In fact, one of the changes that occurs in the skin stroma

during ageing is the increase of senescent dermal fibroblasts which have acquired a senescence-associated secretory phenotype (SASP). SASP components are assumed to create a permissive microenvironment which contributes to tumor progression. However, the mechanisms underlying SASP-induced tumor progression of cSCC are not fully understood in molecular details. As cell motility is a hallmark of tumor progression, we investigated the paracrine SASP effect of senescent fibroblasts on the migration of cSCC lines using transwell migration assay. Conditioned media of senescent fibroblasts significantly increased the migration of cSCC lines in comparison to media conditioned by young fibroblasts. Interestingly, senescent conditioned media was found to be enriched with the chemoattractant protein Chemerin, as shown with ELISA. This finding was correlated with the upregulation of Chemerin transcripts in senescent fibroblasts compared to young fibroblasts. As well, enhanced concentrations of Chemerin protein were detected in human dermal fibroblasts of skin sections of old compared to young individuals using immunofluorescence staining. A complementary approach was used to analyse the chemokine receptors in cSCC lines *in vitro*. Notably, the expression of Chemerin receptor CCRL2 was significantly augmented in all tested cSCC lines compared to normal keratinocytes, confirmed with immunostaining of skin biopsies *in situ* in patients suffering from SCC. Chemerin enhanced the migration of SCC lines in a concentration dependent manner, which was mediated through the activation of the mitogen-activated protein kinase (MAPK) signaling pathway. Inhibition of MAPK signaling pathway using the ERK inhibitor SP600125 and the JNK inhibitor FR180204 significantly impaired SASP-induced migration of tumor cells in response to senescent conditioned media and recombinant human Chemerin. Taken together, these data suggest that senescent fibroblasts may facilitate cutaneous squamous cell carcinoma progression through Chemerin-mediated activation of MAPK pathway in SCC cells.

P248

Sensitization of melanoma cells for the death ligand TRAIL is based on cell cycle arrest, ROS production and activation of proapoptotic Bcl-2 proteins

S. Quast, K. Steinhorst, M. Plötz and J. Eberle *Charité – Universitätsmedizin Berlin, 10117 Berlin, Germany*

The death ligand TRAIL (TNF-related apoptosis-inducing ligand) represents a promising strategy for melanoma due to significant expression of TRAIL receptor 1 in melanoma metastases and high TRAIL sensitivity through this receptor. However, prevalent and inducible resistance are limiting its clinical use. In previous work, we and others have described multiple strategies leading to TRAIL sensitization; however, the common principles of these strategies remained elusive. Here, we demonstrate in melanoma cell lines (TRAIL-sensitive, TRAIL-resistant or TRAILselected cells with acquired resistance) that G1 cell cycle arrest clearly correlates with enhanced TRAIL sensitivity. Cell cycle arrest was induced by cell confluence, serum starvation or a CDK4/6 inhibitor. Addressing the signalling pathways revealed disruption of the mitochondrial membrane potential and production of reactive oxygen species (ROS) in response to the antiproliferative conditions alone. Activation of the proapoptotic Bcl-2 protein Bax and complete inhibition of apoptosis by Bcl-2 overexpression underlined the critical involvement of mitochondrial apoptosis pathways. Most pronounced was the upregulation of small proapoptotic Bcl-2 proteins as the BH3-only protein Puma and Bcl-xS. These data provide a general understanding on TRAIL sensitization, allow a new view on therapeutic strategies by CDK inhibitors and may suggest a selective targeting by cell cycle inhibition and TRAIL.

P249

Modelling genetic heterogeneity as a resistance mechanism to cancer immunotherapy using CRISPR-Cas9

N. Glodde[1], T. Bald[1], D. van den Boorn-Konijnenberg[2], J. Schmid-Burgk[3], V. Hornung[3], T. Tüting[1] and M. Hölzel[2] *[1]Experimental Dermatology, Bonn, Germany; [2]Institute for Clinical Chemistry and Clinical Pharmacology, Bonn, Germany; [3]Institute of Molecular Medicine, Bonn, Germany*

Background: Novel approaches for T-cell immunotherapy and for the inhibition of oncogenic signal transduction pathways have revolutionized melanoma therapy over the last years. However little is known how phenotypic adaptation of melanoma cells and selection of genetic variants contribute to therapy resistance.

Methods: Here we used a multimodal antigen-specific T-cell immunotherapy in a transplantable syngeneic mouse melanoma model to simultaneously compare how adaptive antigen down-regulation and selection of genetic antigen-loss variants contribute to therapy resistance. We generated genetic heterogeneity of an endogenous lineage antigen using the CRISPR-Cas9 genome engineering technology and scrutinized monoclonal versus polyclonal genome editing strategies. Regional genetic heterogeneity was visualized using fluorescent cell labelling to enable tracing of clonal evolutions as well as reciprocal tumour-immune cell interactions in a therapy and genotype dependent manner.

Results: Our results show that wild-type melanoma cells adaptively suppressed target antigen expression at recurrence, but genetic antigen-loss variants were also strongly enriched. This demonstrates that total antigen levels are critical immunotherapeutic determinants and emphasizes the need to assess both the regulation and the genetic heterogeneity of target antigens for personalized cancer immunotherapies.

Conclusion: Taken together, this provided first insights into the dynamic evolution of genetic and non-genetic melanoma heterogeneity in a preclinical therapeutic *in vivo* model of immunotherapy. In the future the CRISPR/Cas9 genome editing technology will enable us to study other clinically relevant genomic aberrations with implications for resistance to immunotherapies.

P250

Deletion of ERBB2 in mouse skin reduces tumorigenesis in multi-stage chemical carcinogenesis

M. Dahlhoff and M. R. Schneider *LMU München, Institute of Molecular Animal Breeding and Biotechnology, Gene Center, 81377 Munich, Germany*

The tyrosine kinase receptor ERBB2 (HER2, neu) is a member of the epidermal growth factor receptor (EGFR, ERBB1, HER1) family, which further includes ERBB3/HER3 and ERBB4/HER4. These receptors are usually activated by ligand binding to the extracellular domain followed by homo- or heterodimerization, which activates the intracellular kinase activity and initiates a downstream signaling cascade. ERBB2- mediated signaling is mainly transduced by the phosphatidylinositol 3-kinase (PI3K)/Akt and the MAPK pathways. Notably, although ERBB2 has no known ligands, its high catalytic activity makes ERBB2 the preferential ERBB dimerization partner. In the skin, ERBB2 is co-expressed with the EGFR in the epidermal basal layer and in proliferative cells of the pilosebaceous unit, and with ERBB3 in the epidermal suprabasal layer. While ERBB2's role in human melanoma and non-melanoma skin cancers remains uncertain, transgenic overexpression of ERBB2 in mice was shown to cause epidermal and follicular hyperplasia and spontaneous tumor formation, and a two-stage skin carcinogenesis experiment suggested that chemical inhibition of ERBB2 may be effective in suppressing tumor promotion. ERBB2 is also activated by UV irradiation and increases UV-induced skin tumorigenesis.

To ablate ERBB2 expression the epithelial compartment of mouse skin, we crossed mice carrying a conditional Erbb2 allele with transgenic animals expressing cre recombinase under the control of the keratin 5 (K5) promoter. Recombination of the Erbb2 allele and loss of ERBB2 in the skin, with unchanged receptor levels in other organs, was confirmed by PCR and immunohistochemistry, respectively. K5Cre;Erbb2del mice were born at the expected ratios and showed no obvious abnormalities, strongly indicating that ERBB2 is dispensable for the development and the homeostasis of the epidermis and its appendages. Next, to analyze the function of ERBB2 during tumorigenesis, we employed a multi-stage chemical carcinogenesis protocol. Seven-week-old K5Cre;Erbb2del females and control littermates received a single application of the initiating agent 7,12-dimethylbenz(a)anthracene followed by multiple applications of the promoting agent 12-O-tetradecanoylphorbol-13-acetate for several weeks. Tumors became visible on the back skin of control mice from the 8th week after DMBA treatment, and 87% of control mice showed at least one tumor by the 14th week. In contrast, in Erbb2del mice, the first tumors appeared only by the 13th week after DMBA, and by the end of the experiment (22 weeks after DMBA application) more than 50% of the mice remained without any visible tumor. The mean number of tumors per mouse increased with time in both groups, but remained significantly lower in Erbb2del (~0.8 tumors/mouse 22 weeks after DMBA) compared to control (2.8 tumors/mouse 22 weeks after DMBA) mice. A similar effect was observed in the mean tumor size, which increased with time in both groups but was considerably smaller in Erbb2del animals (~0.9 mm at 22 weeks after DMBA) compared to control (~2.3 mm at 22 weeks after DMBA) mice. The present data indicate that ERBB2 signaling contributes to tumor growth during multi-stage chemical carcinogenesis in mice.

P251

Cell cycle phase determines efficacy of bortezomib, temozolomide and MAPK inhibitor-induced apoptosis in melanoma

D. S. Hill[1,2], K. A. Beaumont[1,3], S. M. Daignault[4], D. M. Sharp[1,3], B. Gabrielli[4], W. Weninger[1,5] and N. K. Haass[4,5] *[1]The Centenary Institute, Sydney, Australia; [2]Newcastle University, Dermatological Sciences, Newcastle upon Tyne, UK; [3]University of Sydney, Sydney Medical School, Sydney, Australia; [4]The University of Queensland, The University of Queensland Diamantina Institute, Translational Research Institute, Brisbane, Australia; [5]University of Sydney, Discipline of Dermatology, Sydney, Australia*

Many cancer therapies are efficient at inducing cell death in 2D cell culture, but fail in animal models or clinical trials. Understanding what environmental or intrinsic factors alter drug efficacy is thus important for designing therapeutic strategies. Here we have used 2D and 3D culture models and the fluorescence ubiquitination cell cycle indicator (FUCCI) to investigate the effect of environmentally or pharmacologically induced G1 arrest on bortezomib, temozolomide and MAPK pathway inhibitor (MAPKi) cytotoxicity in melanoma. We show that G1-arrested melanoma cells are resistant to bortezomib and temozolomide-induced cell death, which occurs preferentially during G2/M phase, while in contrast G1-arrested cells are more sensitive to MAPKi, which induce apoptosis of cells in G1 phase. The effect of the cell cycle status on drug efficacy therefore has implications on the choice and timing of drug combination therapies, as demonstrated by the increased resistance of melanoma cells to bortezomib or temozolomide-induced cell death following pre-treatment with MAPKi. In addition this study highlights that the tumour microenvironment and proliferative status of cancer cells may influence drug efficacy in solid tumours.

P252

Visualizing the dynamics of melanoma cell phenotypic plasticity in response to inflammatory and hypoxic environmental stimuli

A. Braun, T. Bald, M. Rogava, N. Shridhar and T. Tüting *Laboratory of Experimental Dermatology, Department of Dermatology, University Hospital Bonn, 53127 Bonn, Germany*

Metastatic melanoma is a deadly disease and has remained a therapeutic challenge over the past decades. This project is based on the general hypothesis that aggressive growth, metastatic spread and therapy resistance of melanoma cells is associated with a high degree of phenotypic plasticity, which allows them to rapidly adapt to changing environments. HMGB1 translocation and autophagy are two tightly linked processes utilized by cells to counteract environmental stress and survive. To gain further insights into the molecular and cellular mechanisms underlying the phenotypic plasticity of melanoma cells we study the cytosolic translocation and release of the nuclear protein HMGB1 and the induction of autophagy in melanoma cells that are exposed to inflammatory and hypoxic environmental conditions. By employing our ligationindependent cloning technique we created plasmids encoding HMGB1-TagGFP2, LC3B-TagRFP and H2B-TagBFP transgenes in our retroviral vector backbone and subsequently created retroviral particles. By transfection of the HCmel12 melanoma cell line we generated cells stably expressing the HMGB1, LC3B and H2B transgenes. First stress experiments clearly show the functionality of the constructs by nuclear location of HMGB1 and clustering of LC3B upon activation of autophagy with 100 μM Rapamycin. It will be of great interest to study the intersection between autophagy induction and melanosome fate when melanoma cells downregulate their melanocytic differentiation program in response to inflammatory or hypoxic stimuli. This work provides the basis to further explore the role of HMGB1 translocation and autophagy induction for melanoma cell phenotypic plasticity in response to environmental stress *in vivo* using confocal and intravital microscopy techniques.

P253

Expression and function of Nrf2 in human melanoma cells

M. Apel, D. Metze, N. Weiss and M. Boehm *University of Muenster, Department of Dermatology, 48149 Muenster, Germany*

Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a key transcription factor that regulates expression of phase II antioxidant enzymes including heme oxygenase-1 (HO-1), gamma-glutamylcysteine synthetase (gamma-GCS), glutathione-Stransferase Pi (GSTPi) and quinone oxidoreductase 1 (NQO1). Nrf2 has been found to be upregulated in some tumor entities resulting in increased tolerance against oxidative stress and cell death. As a consequence chemosensitivity against cytoreductive therapies is reduced. With regard to melanoma little is known about the function and regulation of Nrf2. Previously we found that Nrf2 is regulated in normal human melanocytes by UVB irradiation and alpha-melanocyte-stimulating hormone. In normal human skin Nrf2 was largely absent in epidermal melanocytes as shown by immunohistochemistry. Here we show that Nrf2 is upregulated in a large proportion of human melanoma cell lines at RNA and protein level compared to normal melanocytes. Activated (phosphorylated) Nrf2, however, was detectable in the nuclei of both normal melanocytes and melanoma cells *in vitro* with no apparent difference. Gene knock-down of Nrf2 by siRNA resulted in suppression of the Nrf2- dependent enzymes HO-1, NQO1, GSTPi and gamma-GCS and increased hydrogen peroxide- or etoposide-mediated cell death in melanoma cells indicating a prosurvival effect of Nrf2 *in vitro*. Next we performed immunohistochemical analysis of cutaneous melanoma samples ($n = 46$) employing antibodies against total Nrf2 and phosphorylated Nrf2. In accordance with previous findings total and nonphosphorylated Nfr2 was under the detection limit in epidermal melanocytes *in situ* in healthy human skin. Total Nrf2 immunoreactivity in melanoma samples was variable. In advanced stage melanomas (Clark IV-V) and metastases all samples displayed either clear-cut positive Nrf2 immunoreactivity, weak or heterogeneous staining with individual tumor cells being immunoreactive and non-reactive within the same specimen. In early melanomas (Clark I and II-III), on the other hand, a significant proportion of melanomas did not display total Nrf2 immunoreactivity within tumor cells. *In situ* immunoreactivity for phosphorylated Nrf2 was confined to the nuclei of melanoma cells. There was a trend towards increased immunostaining in advanced melanomas compared to Clark I melanomas while staining in metastases was very variable. Our findings provide a first insight into the expression and possible function of Nrf2 in melanoma. Further studies are needed to clarify the function of Nrf2 with regard to cell survival, chemosensitivity and tumor progression in melanoma.

P254

Phospho-proteomic analysis reveals increased CK2alpha kinase activity in NRAS (Q61) mutant melanoma

C. Posch[1,2], M. Sanlorenzo[1,3], I. Vujic[1,2], J. Oses-Prieto[4], S. Kim[1], J. Ma[1], K. Lai[1], M. Zekhtser[1], G. Green[1], R. Esteve-Puig[1], A. Burlingame[4], K. Rappersberger[2] and S. Ortiz-Urda[1] *[1]University of California San Francisco, Dermatology, San Francisco, USA; [2]The Rudolfstiftung Hospital, Dermatology, Vienna, Austria; [3]University of Turin, Dermatology, Turin, Italy; [4]University of California San Francisco, Pharmaceutical Chemistry, San Francisco, USA*

Background: Mutations in the NRAS oncogene are among the most frequent driving alterations in cutaneous melanoma. Single nucleotide changes are predominantly found in codon 12 and 61, impairing the intrinsic catalytic activity of NRAS, thus preventing physiological cycling of the protein. Mutant, and thereby constantly active NRAS contributes to tumor initiation, growth, invasion and metastasis, still it has yet been impossible to pharmaceutically target this protein. Even though mutations in codon 12 and 61 can both be considered activating mutations, each mutation has been recognized to affect protein function in very distinct ways; however, little is known about potential differences in signaling resulting from these alterations.

Methods: To investigate signaling changes of different NRAS mutations we analyzed the phosphoproteom of primary human melanocytes transduced with NRAS(G12), NRAS(Q61) or empty vector controls using stable isotope labeling by amino acids in cell culture (SILAC), titanium dioxide phosphopeptide enrichment, and phospho- Y immunoprecipitation followed by mass spectrometry. Additional analysis included a phosphorylation-motif search, kinase prediction analyses, immunoblotting, and immunohistochemistry of patient samples.

Results: SILAC followed by mass spectrometry identified 14155 spectra of 3371 unique phosphopeptides mapping to 1159 proteins (FDR<2%). Data revealed pronounced PI3K/AKT signaling in NRAS(G12V) mutant cells and pronounced mitogen activated protein kinase (MAPK) signaling in NRAS(Q61L) variants. Kinases involved in phosphorylating the specific sites detected by SILAC indicated distinct clusters for NRAS(G12V) and NRAS(Q61L) mutant cells. CK2alpha kinase was significantly overrepresented in PHM bearing NRAS(Q61L) mutations. Similar differences were found in human NRAS mutant melanoma cell lines. NRAS(Q61) lines were more sensitive to pharmacologic CK2alpha inhibition compared to NRAS(G12) mutant cells. Furthermore, CK2alpha showed higher expression in clinical NRAS(Q61) mutant melanoma samples at protein and mRNA levels.

Conclusions: The preclinical findings of this study indicate that codon 12 and 61 mutant NRAS cells have distinct signaling characteristics. Data reveal that CK2alpha kinases are hyper-activated in NRAS (Q61) mutant melanoma cells. Since CK2alpha inhibitors are readily available for clinical applications, this study provides new insight into the potential therapeutic targeting of CK2alpha in NRAS mutant melanoma patients.

P255

Ultraviolet (UV) – a irradiation induces melanoma invasion via enhanced Warburg effect

T. Baban[1], Y. Kamenisch[2], T. Sinnberg[1], W. Schuller[1], A. von Thaler[1], G. Metzler[1], B. Schittek[1], C. Garbe[1], J. Bauer[1], M. Röcken[1] and M. Berneburg[2] *[1]Eberhard Karls University, Department of Dermatology, 72076 Tuebingen, Germany; [2]Universitätsklinikum Regensburg, Department of Dermatology, 93053 Regensburg, Germany*

Melanoma is a malignant tumor for which exposure to ultraviolet (UV) radiation is considered to be an important risk factor. Especially UVA (320–400 nm) radiation induces the formation of reactive oxygen species (ROS) which oxidatively damage cellular molecules. It was recently shown that UVA radiation is capable to induce murine melanoma, but the role of UVA in the progression of melanoma is still not investigated. During early progression of melanomas before metastasising, most melanomas show initial proliferation of melanoma cells and a metabolic characteristic of most proliferating tumor cells is the preference of aerobic glycolysis instead of oxidative phosphorylation (Warburg effect). Here we investigated the role of repetitive UVA radiation in progression of melanoma, especially induction of progression markers, changes in Warburg effect and invasive potential. In skin reconstructs treated with repetitive UVA irradiation initial melanoma cells show increased initial dermal invasion. Upon UVA radiation, initial melanoma cells show increased Warburg effect with increased glucose consumption and increased lactate production. The tumor marker transketolase and phosphorylated Akt kinase, which are involved in metabolic changes and associated with proliferation, are also elevated upon UVA radiation. With *in vitro* invasion assays we show, that lactate, which is produced via UVA enhanced Warburg effect, increases invasiveness of initial melanoma cells. This effect is mediated by reactive oxygen species which are induced by UVA radiation as treatment with ROS scavengers impairs UVA induced lactate production and invasion. The expression of tumor relevant matrix metalloproteinases (MMP) and urokinase type plasminogen activator (uPA) are highly upregulated upon UVA irradiation or treatment with lactic acid and MMP and uPA mainly facilitate *in vitro* invasion. Furthermore treatment with lactic acid alone can mimic the UVA induced increase of the invasive potential *in vitro*. Therefore we could show in melanoma cells, derived from melanomas of early progression that production of lactate, induced by UVA radiation, increases invasiveness of initial melanoma cells via expression of MMPs.

P256

Emerging role for cell type specific VEGF expression at the crossroads of HPVmediated carcinogenesis and wound healing

X. Ding[1], T. Lucas[1], G. Marcuzzi[2], H. Pfister[2] and S. A. Eming[1,3] *[1]University of Cologne, Department of Dermatology, 50937 Cologne, Germany; [2]University of Cologne, Institute of Virology, 50937 Cologne, Germany; [3]University of Cologne, Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), 50937 Cologne, Germany*

Aberrant wound healing and tissue regeneration can lead to carcinogenesis. Our previous study demonstrated that macrophage-derived Vascular endothelial growth factor-A (VEGF-A) plays a critical role in coordinating effective tissue growth and vascularization during the wound healing response after excisional skin injury. Emerging evidence suggests a central role for VEGF-A in regulating tumor development through both induction of tumor angiogenesis but also via angiogenesis-independent mechanisms. Detailed mechanisms of angiogenesisindependent effects of VEGF-A in carcinogenesis remain elusive. The role of VEGFA in human papillomaviruses (HPV)-induced non-melanoma skin cancer (NMSC) has not been resolved, neither the question whether diverse cellular sources of VEGF-A may impact this process.

There is strong clinical evidence that the genus beta human papillomaviruses (HPV) are involved in NMSC development in patients with epidermodysplasia verruciformis (EV). However, the mechanism of action remains a challenge. EV might serve as model disease to gain an overall better molecular understanding of HPV-mediated carcinogenesis. We previously developed a transgenic mouse line expressing the complete early genome region (CER) of HPV8 under the control of human keratin14 (K14) promoter. HPV8 mice recapitulate the HPV-induced SCC pathology and have been proven to be a valuable *in vivo* model to unravel the molecular pathology of HPV-induced skin cancer.

In this study we dissected the contribution of epidermis- versus myeloid cell-derived VEGF-A in HPV8-mediated skin cancer using a combination of HPV8 transgenic mice and conditional gene targeting for VEGF-A. We show, that epidermis-specific deletion of VEGF-A results in complete abrogation of tumor initiation in HPV8 mice both spontaneous and in the presence of diverse tumor promoting conditions. In contrast, myeloid cell-derived VEGF-A is only critical in wound-induced tumorigenesis triggered by full thickness excision skin injury. Mechanistically, we show that blocking VEGFR2 inhibited injury-induced papilloma formation in HPV8 transgenic mice, indicating an important paracrine function of VEGF-A on tumor angiogenesis. Furthermore, our findings provide evidence that epidermal HPV8 proteins can deviate a primarily beneficial and healing-promoting acute inflammatory response into a sustained inflammatory response leading to hyperplastic growth, and that myeloid cell-derived VEGF-A plays a critical role in this process. Interestingly, reduced clonal growth of VEGF-A depleted keratinocytes *in vitro* could not be rescued by external rVEGF-A, suggesting an additional cell-autonomous activity of VEGF-A in keratinocytes, independent from angiogenesis. Gene expression analysis and IHC staining suggest an autocrine mechanism mediated by VEGFR1 and Nrp1. Taken together, here we provide novel mechanistic insights in distinct functions of epidermal-versus myeloid cell-derived VEGF-A in HPV8-mediated tumor development, which may have important implications for the prevention and treatment of HPV-mediated skin cancer.

P257

Casein kinase 1α has a dominant role in the biology of malignant melanoma within the CK1 familiy

T. Sinnberg, J. Wang, B. Sauer and B. Schittek *Eberhard-Karls-Universität Tuebingen, Dermatology, 72076 Tuebingen, Germany*

Background: We previously identified CK1α as a novel tumor suppressor in melanoma and reported that the loss of CK1α leads to increased proliferation and invasive growth of melanoma cells by strong activation of the Wnt/β-catenin signaling pathway.

Methods: In this study we analyzed expression and the functional effects of the CK1- isoforms α, δ and ε dominantly expressed in melanoma cells by quantitative real-time PCR, western blot and immunohistochemistry. We downregulated CK1 kinase activity with isoform specific siRNAs and small molecule inhibitors. Vice versa we overexpressed the CK1 isoforms alpha, delta and epsilon using viral vectors and tested the biological effects on melanoma cell proliferation, migration and invasion. We further correlated a CK1α associated gene expression pattern which we generated in our previous study to the suvival data of melanoma patients in the TCGA database.

Results: We show that protein expression of all three CK1-isoforms is downregulated in metastatic melanoma cells compared to benign melanocytic cells. Furthermore, the CK1δ and ε isoforms negatively regulate expression of each other, whereas CK1α expression is independently regulated in melanoma cells. Inhibition of the expression and activity of CK1δ or CK1ε by specific inhibitors or siRNAs had no significant effect on the growth and survival of metastatic melanoma cells. Moreover, the overexpression of CK1δ or CK1ε in melanoma cells failed to induce cell death or cell cycle arrest although p53 signaling was activated. This is in contrast to the effects of CK1α where the up-regulation induces cell cycle arrest and apoptosis in metastatic melanoma cells. Finally, an CK1α activity associated gene expression pattern was significantly correlated to the overall survival of melanoma patients in the TCGA database.

Conclusion: These data indicate that CK1α has a dominant and non-redundant function in melanoma cells and that the CK1δ and ε isoforms are not substantially involved in melanoma progression.

P258

Characteristics of exosomes derived from viral associated Merkel cell carcinoma

S. Jansen[1], K. Fan[1], M. Stiller[2], S. Seide[1], C. Ritter[2] and J. C. Becker[1,2] *[1]Translational Dermato-Oncology, 45147 Essen, Germany; [2]German Consortium for Translational Cancer Research (DKTK/DKFZ), 45147 Essen, Germany*

Merkel cell carcinoma (MCC) is a highly aggressive neuroendocrine skin cancer with neuroendocrine differentiation. At least 80% of MCC-tumors are positive for the Merkel cell polyomavirus (MCPyV) genome, which is clonally integrated into the host genome. The MCPyV early genes encode for the transforming large (LTA) and small tumor antigen (sTA). The tumor microenvironment plays a major role in cancer in general and in viral cancerogenesis in particular. The tumor consist not only of transformed cells, but also accommodate high numbers of non-transformed cells with different origin, e.g. stromal, endothelial and immune cells. All these cells are orchestrated by the transformed cells to promote tumor progression. A potent way to impact surrounding cells is a horizontal transfer of (oncogenic) material like nucleic acids (e.g. mRNA, miRNA, DNA), proteins or peptides via exosomes. These small vesicles are 50–100 nm in size. They originate from the endosomal machinery within a cell by inward budding of multivesicular bodies (MVBs), which fuse with the cell membrane to release the exosomes. Exosomes were enriched from media of well established MCC cell lines (WaGa and PeTa) by sequential ultrafiltration and -centrifugation steps. The enriched exosomes expressed typical exosomal marker proteins such as CD63 as detected by immunoblot. Analysis of the exosomal content by means of real-time PCR revealed the presence of sTA and LTA mRNA, as well as the microRNA 375. Surprisingly, the MCC-derived exosomes also contained circular DNA comprising the LTA and sTA sequences as detected by rolling circle amplification (RCA).

In summary, we have shown for the first time the release of exosomes containing mRNA and DNA coding for transforming early genes by established MCC cell lines. Currently we are analyzing the exosomal DNA by next generation sequencing and started to scrutinize their effect on immune cells by use of EGFP-marked exosomes.

P259

MITF and c-Jun antagonism interconnects melanoma dedifferentiation with pro-inflammatory cytokine responsiveness and myeloid cell recruitment

S. Riesenberg[1], J. Reinhardt[1], I. Davidson[2], C. R. Goding[3], G. Jönsson[4], J. Landsberg[5], T. Tüting[5] and M. Hölzel[1] *[1]University Hospital Bonn, Unit for RNA Biology, 53127 Bonn, Germany; [2]Institut de Génétique et de Biologie Moléculaire et Cellulaire, Department of Functional Genomics and Cancer, 67404 Illkirch, France; [3]University of Oxford, Ludwig Institute for Cancer Research, OX3 7DQ Oxford, UK; [4]Department of Clinical Sciences, Division of Oncology and Pathology, 221 85 Lund, Sweden; [5]University Hospital Bonn, Laboratory for Experimental Dermatology, 53127 Bonn, Germany*

Inflammation promotes phenotypic plasticity of melanoma cells, a source of nongenetic heterogeneity that contributes to metastasis and therapy resistance. However, the molecular framework linking pro-inflammatory signals with melanoma phenotype switching is poorly understood. We used bioinformatic and functional genomic approaches and identified a reciprocal antagonism between the melanocyte lineage transcription factor MITF and the AP-1 component c-Jun. This interrelationship connects inflammation-induced dedifferentiation with proinflammatory cytokine responsiveness of melanoma cells, favouring myeloid cell recruitment into the tumour. The AP-1 transcription factor complex is known to synergize with NF-KB in the transcriptional response to inflammation. We found that induction of c-Jun by the major pro-inflammatory cytokine TNF-α was critical to instigate a gradual loss of MITF expression. Integration of ChIP-seq data revealed MITF binding sites within the regulatory regions of the c-Jun genomic locus and suggested a direct negative regulation of c-Jun by MITF, as reducing MITF levels unleashed c-Jun expression. c-Jun upregulation in turn amplifies TNF-stimulated cytokine expression and is required for TNF-induced MITF suppression. Together, this molecular cascade constituted a potent feed-forward mechanism that turned poor peak-like transcriptional responses to TNF-α into a strong, progressive and persistent induction of cytokines and chemokines in melanoma cells. Given that chemokines are critical determinants for immune cell recruitment into the tumor microenvironment, we hypothesized that the identified antagonism between MITF and c-Jun may define specific alterations of the microenvironmental immune cell compartment. Indeed, dedifferentiated MITFlow/c-Jun-high human melanomas exhibited a preferential

myeloid cell infiltration. Therefore, patients with MITFlow/c- Jun-high melanomas may benefit from myeloid cell-directed therapies that counteract their growth-promoting and immunosuppressive functions.

P260

Role of the aryl hydrocarbon receptor pathway in melanoma pathogenesis

M. Mengoni, N. Shridhar, A. Braun, T. Bald, E. Gaffal and T. Tüting *Laboratory of Experimental Dermatology, Department of Dermatology and Allergy, University Hospital of Bonn, 53127 Bonn, Germany*

Background: The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor which helps to orchestrate cellular responses to various environmental stimuli. AhR-mediated signaling has been shown to play a role in infectious, inflammatory and malignant diseases as well as in developmental processes including cellular proliferation and differentiation.
UV radiation is the major environmental risk factor for the development of malignant melanoma and contributes to disease progression and metastatic spread. Activation of the AhR in melanocytes mediates the response to UV light, e.g. pigmentation and cell proliferation. We hypothesize that the AhR pathway attenuates melanoma cell responses to inflammatory and DNA damage-induced stress, promoting their adaptation to a changing microenvironment during disease progression. Thereby it may support tumor cell survival and metastatic spread. In this context, we examined the potential role of the AhR pathway in melanoma initiation and progression *in vitro* and *in vivo* using the transplantable murine melanoma cell line HCmel12.
Methods: To study the effects of AhR-mediated signaling upon inflammatory stimuli on proliferation, migration and cellular stress responses we first generated AhR-deficient (AhR−/−) melanoma cells employing the CRISPR/Cas9-based genome editing technology. To regain AhR function in these cells, we then stably overexpressed the receptor using a retroviral vector.
We stimulated AhR-deficient and -reconstituted cells with TNF-α and LPS in a set of different doses and analyzed their impact on proliferation and differentiation between 24 h and 72 h post-challenge. Wild-type HCmel12 cells served as control.
In syngeneic mouse transplantation experiments we investigated the impact of AhR signaling in the microenvironment on progressive growth and metastatic spread *in vivo*. The AhR repressor (AhRR) is an AhR target gene which represses AhR signaling via a negative auto-regulatory feedback mechanism. We injected HCmel12 wild-type cells s.c. into AhRR-deficient and -competent mice and studied tumor growth kinetics and formation of metastases.
Results: First *in vitro* data revealed a low basal expression of Ngfr (CD271) in AhR−/− melanoma cells compared to parental wild-type cells. In line with these findings we found a markedly decreased upregulation of Ngfr after 24 h stimulation with TNF-α by flow cytometric and immunoblot analyses. AhR−/− cell lines showed no responsiveness to LPS in the same period of time in contrast to dedifferentiating, Ngfr-elevated wild-type cells.
In first trials we observed tendencies of accelerated tumor growth and increased numbers of lung metastases in AhRR-deficient mice compared to competent mice *in vivo*. Flow cytometric analyses also showed a higher infiltration of neutrophils in AhRR-deficient mice.
Conclusion: Taken together, our work provides evidence that AhR-mediated signaling is involved in melanoma cell responses to inflammatory stress and in cell differentiation. Our experiments demonstrate a relevant role for an intact AhR pathway for phenotypic plasticity of melanoma cells and contribution to adaption of melanoma cells to changing environments during disease progression.

P261

A malignant peripheral nerve sheath tumor-like melanoma phenotype orchestrates mast cell recruitment

J. Landsberg[1], M. Hölzel[2], N. Glodde[1], T. Bald[1], M. Rogava[1], A. Becker[3], G. Jönsson[4] and T. Tüting[1] *[1]University of Bonn, 1Laboratory of Experimental Dermatology, Department of Dermatology and Allergy, 53127 Bonn, Germany; [2]University of Bonn, Unit for RNA Biology, Department of Clinical Chemistry and Clinical Pharmacology, 53127 Bonn, Germany; [3]University of Bonn, Section of Translational Epileptology, Department of Neuropathology,, 53127 Bonn, Germany; [4]Lund University, Department of Oncology and Pathology, Clinical Sciences, SE-221 00 Lund, Sweden*

Human melanomas show considerable variations in genetic changes, cell morphology and in microenvironmental composition. Genetically engineered mice have successfully been used to model the impact of genomic aberrations involved in melanoma pathogenesis. However, it is unclear whether they recapitulate the phenotypic heterogeneity of human melanoma cells and the complex interactions with the immune system. Here we report the unexpected finding that immune-cell poor pigmented and immune-cell rich amelanotic melanomas develop simultaneously in Cdk4R24C mutant mice upon melanocyte-specific conditional activation of oncogenic BrafV600E and a single application of the carcinogen DMBA. Interestingly, amelanotic melanomas showed morphological and molecular features of malignant peripheral nerve sheath tumors (MPNST). A bioinformatic cross-species comparison using a gene expression signature of MPNST-like mouse melanomas identified a subset of human melanomas with a similar histomorphology in the TCGA database. Exploring their transcriptional immune cell subtype compositions we found a highly significant association with mast cells. Importantly, mouse MPNST-like melanomas were also extensively infiltrated by mast cells and expressed mast cell chemoattractants similar to their human counterparts. A transplantable mouse MPNST-like melanoma cell line recapitulated mast cell recruitment in syngeneic mice demonstrating that this cell state can directly orchestrate histomorphology and microenvironmental composition. Our study emphasizes the importance of reciprocal, phenotype-dependent melanoma-immune cell interactions and argues for a critical role of mast cells in a subset of melanomas. We further conclude that our BrafV600E-Cdk4R24C model will facilitate the development of cell state-selective and microenvironment-directed therapies as it recapitulates at least two distinct human melanoma phenotypes at once.

P262

Chemical-induced neutrophilic inflammation promotes metastatic spread of melanoma

T. Bald, J. Landsberg, P. Jansen, E. Gaffal and T. Tüting *Laboratory for Experimental Dermatology, Department of Dermatology and Allergy, 53105 Bonn, Germany*

Increased neutrophil counts locally in the tumor tissue as well as in the peripheral blood correlate with poor clinical outcome in melanoma patients suggesting a pro-tumorigenic role of neutrophils for the pathogenesis of malignant melanoma. Recently, we discovered that neutrophil-driven skin inflammatory responses induced upon repetitive UV-irradiation selectively promote metastatic spread of incipient cutaneous melanomas in genetically engineered Hgf-Cdk4 (R24C) mice. We hypothesized that other pro-inflammatory stimuli that induce neutrophilic inflammatory responses also promote the development and progression of melanomas. In the present study, we investigated how the most potent and frequently used tumor promoter 12-O-Tetradecanoylphorbol-13-acetate (TPA) affects the development and progression of carcinogen-induced and transplanted Hgf- Cdk4 (R24C) melanomas. Local and systemic neutrophilic inflammatory responses induced by TPA-treatment also selectively increase the metastatic spread of melanoma cells to draining lymph nodes and lungs. Using a highly metastatic Hgf-Cdk4 (R24C) melanoma skin transplant we could show that a TPA-induced neutrophilic inflammation enhances systemic spread of melanoma cells which was depended on intact TLR4 signalling in recipient mice and on the presence of neutrophils. Altogether, our experimental results support an important mechanistic role of TLR4-driven neutrophilic inflammation for melanoma progression.

P263

CD73 correlates with an inflammatory mesenchymal cell state in melanoma and is regulated via MAPK signaling

J. Reinhardt[1], J. Landsberg[2], S. Riesenberg[1], J. Schmid-Burgk[3], D. Nettersheim[4], D. van den Boorn[1], T. Tüting[2] and M. Hölzel[1] *[1]University Hospital Bonn, Institute of Clinical Chemistry and Clinical Pharmacology, 53127 Bonn, Germany; [2]University Hospital Bonn, Laboratory of Experimental Dermatology, Department of Dermatology and Allergy, 53127 Bonn, Germany; [3]University Hospital Bonn, Institute of Molecular Medicine, 53127 Bonn, Germany; [4]University of Bonn, Department of Developmental Pathology, 53127 Bonn, Germany*

Background: CD73 is a cell surface 5′ ectonucleotidase expressed by melanoma and immune cells that converts extracellular AMP to immunosuppressive adenosine and hence represents a promising new immunotherapeutic target. However, its regulation in melanoma is unknown and we reasoned that it is a critical determinant for clinical strategies.
Methods: We used an integrative approach of global gene expression analysis, pharmacological and genetic perturbations as well as FACS-based cell state characterization.
Results: Gene set enrichment analysis of melanoma cell line panels revealed that CD73 levels correlate with a dedifferentiated mesenchymal phenotype driven by inflammatory and mitogenic signaling. We found that the melanocytic growth factor HGF and the proinflammatory cytokine TNF-alpha synergistically induced CD73 in a MEKERK signaling dependent manner. Consistently, many melanoma cell lines with activating mutations in BRAF or NRAS exhibited high basal CD73 expression that was robustly suppressed by the treatment with BRAF or MEK inhibitors. In line, CD73 levels were restored in BRAF inhibitor resistant cells generated by CRISPR/Cas9-mediated deletion of the negative RAS regulator and tumor suppresser NF1 (neurofibromatosis 1). Using a genetically engineered mouse model, we previously showed that murine melanomas resist T-cell based immunotherapy by inflammationinduced dedifferentiation. Now we demonstrate that these relapse tumors express high levels of CD73 in contrast to untreated controls and cell cultures established thereof had high inflammatory and mitogenic signaling activity.
Conclusions: Our findings link immunosuppressive CD73 expression by melanoma cells to oncogenic MEK-ERK signaling and further support the rationale to combine BRAF inhibitors with immune checkpoint blockade.

P264

Nuclear RAGE drives genomic instability and thereby affects melanoma development, growth and progression

M. Reith[1,2], W. Gebhardt[3], K. Tarnanidis[1,2], V. Schuermans[1,2], K. Ikenberg[4], C. Kehrel[1,2], N. B. Wagner[1,2], J. Utikal[1,2] and C. Gebhardt[1,2] *[1]German Cancer Research Center (DKFZ), Skin Cancer Unit, 69120 Heidelberg, Germany; [2]University Hospital Mannheim, Department of Dermatology, Venereology, and Allergology, 68167 Mannheim, Germany; [3]Institute of Molecular Biology (IMB), Mainz, Germany; [4]University of Zurich (UZH), Institute of Surgical Pathology, Zurich, Switzerland*

The receptor for advanced glycation end-products (RAGE) is known to act as a central driver of tumorigenesis by sustaining a chronic inflammatory tumor microenvironment. Until to date, RAGE has been exclusively described as a cell surface receptor being activated upon engagement with its various extracellular ligands, e.g. S100B, S100A8/A9, HMGB1, and others. This study aimed at elucidating the functional role of RAGE and its isoforms depending on their subcellular distribution in the context of melanoma development, growth and progression. Therefore, various *in vitro* models using melanoma cells or melanocytes and melanoma mouse models as well as tissue-microarrays representing human specimens of malignant melanoma and benign nevi were applied.
The expression analyses revealed an overexpression of RAGE in melanoma cells compared to melanocytes/nevocytes. Moreover, RAGE protein was found to be localized primarily in the nucleus of melanocytes/nevocytes whereas a predominant cell surface/cytoplasmic localization of RAGE is observed in melanoma cells. Nuclear translocation of RAGE depends on the nuclear transport machinery and site-directed mutagenesis of predicted DNA binding sites within the RAGE protein was applied in order to study its functional role in the nucleus. Furthermore, knockdown of RAGE indicated its central function in apoptosis induction.
In conclusion, RAGE and its isoforms are overexpressed and aberrantly localized in malignant melanoma cells compared to melanocytes/nevocytes. Our data point towards a novel function of RAGE in melanoma development depending on its nuclear localization.

Miscellaneous

P265

Ceramide synthase 4 is involved in epidermal barrier maintenance

F. Peters[1,2], S. Brodesser[3], J. C. Brüning[4,5], M. Krönke[2,3] and C. M. Niessen[1,3] *[1]University of Cologne, Department of Dermatology, 50937 Cologne, Germany; [2]University of Cologne, Institute for Medical Microbiology, Immunology and Hygiene, 50935 Cologne, Germany; [3]University of Cologne, Cologne Excellence Cluster on Cellular Stress Responses in Aging Associated Diseases (CECAD), 50931 Cologne, Germany; [4]Max Planck Institute, for Metabolism Research, 50931 Cologne, Germany; [5]University of Cologne, Center for Endocrinology, 50937 Cologne, Germany*

Ceramides are essential constituents of mammalian membranes and key players in different signaling pathways in addition to being central components of the lipid envelope surrounding corneocytes. Ceramide production depends on ceramide synthases (CerS) the family of which consists of six members (CerS1-6), five of which, CerS2-6, are expressed in skin. Although CerS3 was shown to be essential for epidermal barrier formation, little is known on the role of other CerS in epidermal barrier formation and maintenance. As CerS4 is highly expressed in adult epidermis we investigated the role for CerS4 in epidermal barrier maintenance using CerS4 knockout mice. Newborn CerS4-deficient mice showed no obvious changes in epidermal barrier function in line with only low levels of CerS4 epidermal expression at this time point and only minor changes in skin lipid composition. However at later stages inactivation of CerS4 lead to an altered production of epidermal, stratum corneum and sebaceous lipids and ultrastructural analysis revealed a change in lamellar body size and architecture. Additionally, an age dependent increase in the thickness of the interfollicular epidermis was detected, accompanied with changes in terminal differentiation of keratinocytes in CerS4-deficient epidermis. Finally, corneocyte structure was affected with corneocytes being smaller and less circular. The epidermis specific deletion of CerS4 recapitulates the age dependent increase in the thickness of the interfollicular epidermis in whole body knockout mice, indicating an epidermis intrinsic function of CerS4. Thus, our data indicate that CerS4-dependent lipid production controls the maintenance of epidermal barrier function and may regulate terminal differentiation of keratinocytes. As alterations in ceramide contents are associated with a number of diseases such as atopic dermatitis and psoriasis, an understanding of the influence of CerS4-dependent lipid production in epidermal barrier formation is essential to envision new therapeutic approaches.

P266

Hyaluronan-based cryogels are effective scaffolds for human dermal fibroblasts supporting long term growth and matrix deposition *in vitro*

S. Thönes[1,2], L. Kutz[1,2], S. Oehmichen[3], J. Becher[2,4], M. Schnabelrauch[2,4], S. Reichelt[3] and U. Anderegg[1,2] *[1]Leipzig University, Department of Dermatology, 04103 Leipzig, Germany; [2]Collaborative*

Research Center (SFB-TR67) Matrixengineering, Leipzig and Dresden, Germany; [3]Leibniz Institute of Surface Modification, 04318 Leipzig, Germany; [4]INNOVENT e.V., Biomaterials Department, 07745 Jena, Germany

Introduction: Hyaluronan (HA), a native extracellular matrix component, is an attractive starting material to generate cell scaffolds in tissue regeneration. However, the formation of dimensionally and mechanically stable scaffolds with defined inter-connective porosity is hampered by the high solution viscosity of HA even at low concentrations, its high swellability and susceptibility against degradation.
A promising approach to stable and biocompatible scaffolds avoiding cytotoxic reagents is the electron-beam initiated cryogel formation using (meth)acrylated polysaccharides. Here we present data on the fabrication, characterization and biological compatibility of HA-cryogels for the culture of primary human dermal fibroblasts with the perspective to develop a skin compatible scaffolds.
Materials and Methods: Two HA acrylates (DS = 0.8 and 1.0, resp., MW = 70–90 kDa) were synthesized by phase-transfer catalyzed acylation of HA with acryloyl chloride [1]. Cryogels were prepared as described by Reichelt et al.[2]. Briefly, 2–3 wt.-% aqueous solutions of HA acrylates were degassed and frozen in centrifuge tubes at −20°C for 2 h. The irradiation of 12 kGy total was applied in 3 kGy steps using a 10 MeV LINAC at −17°C. The cryogels were characterized with respect to mechanical and swelling properties, gel content and thermal stability. For biological testing the cryogels were seeded with primary foreskin fibroblasts and cultured with continuous shaking. The cells were investigated concerning cell proliferation, gene expression and matrix deposition within the cryogels by XTT assay, quantitative qPCR and (immuno)histochemical staining techniques respectively.
Results and Discussion: Thin cryogel films (2 mm thick) were successfully synthesized by electron-beam initiated cross-linking. The degree of swelling and the gel content were determined to be 43 and 80%, resp. The pore size was in the typical range (50–70 μm) [2].
We established an effective seeding protocol enabling the growth of huFb for at least 28 days *in vitro*. The cell proliferation was as effective as on collagen-coated reference surfaces during the first 48 h. Gene expression analysis demonstrated that huFb were regulated by TGFβ and PDGF with induction of known target genes like collagen I(αI) or HA synthase 2 suggesting an adequate physiological behavior of the cells grown within the cryogels. Histochemical analysis demonstrates that Fb settled the cryogels completely and deposited their own matrix of collagens and glycosaminoglycans. huFb were viable after 4 weeks of culture and the cryogel structure remained stable.
Conclusions: Cryogels were prepared by an electron-beam initiated crosslinking reaction of HA acrylates omitting the use of potentially toxic reagents. The morphology and mechanical properties of the obtained cryogels may be tailored by variation of the composition of the HA derivatives (e. g. degree of acrylation) and the reaction parameters (e. g. polymer concentration).
The cryogels are very effective growth substrates for huFb which proliferate and deposit their own matrix within the pores. Taken together, HA-based cryogels are promising biomaterials for the development of pre-seeded tissue equivalents.

1. Becher, J. et al. Carbohydr. Polym. 93, 438–441 (2013).
2. Reichelt, S. et al. Mater. Sci. Eng. C 35, 164–170 (2014).

P267

Psoriasis-associated changes in the cutaneous microbiome can be identified using next generation sequencing combined with MALDI-TOF spectrometry

E. Langan[1,2], A. Künstner[2,3], D. Thaci[1], D. Zillikens[2,4], S. Ibrahim[2,4], W. Solbach[5,6] and J. Knobloch[5,6]
[1]University of Luebeck, Comprehensive Centre for Inflammation Research, Luebeck, Germany; [2]University of Luebeck, Department of Dermatology, Luebeck, Germany; [3]Max-Planck-Institute of Evolutionary Biology, Evolutionary Genomics Group, Plön, Germany; [4]University of Luebeck, Luebeck, Germany; [5]University of Luebeck, Institute of Medical Microbiology and Hygiene, Luebeck, Germany; [6]German Centre for Infection Research, Luebeck, Germany

Dysregulation of the human microbiome, with subsequent dysbiosis, is postulated to be an important patho-physiological event in the development of several systemic inflammatory diseases. The extent to which the cutaneous microbiome is altered in psoriasis awaits definitive clarification.
In order to address this question, skin swabs/washings were obtained from several skin locations, including the typical psoriasis predilection sites, from patients with moderate to severe plaque-type psoriasis ($n = 14$) and corresponding sites in healthy controls ($n = 9$). In addition, in patients with psoriasis, both lesional and non-lesional skin was swabbed. Following microbial DNA extraction, 16S ribosomal PCR and next generation sequencing (NGS) was used to identify the composition of the cutaneous microbiome. In addition, MALDI-TOF spectrometry was employed to rapidly identify bacteria which were cultured from the skin swabs (culturome) in these and in an additional six patients with plaque-type psoriasis. The use of systemic antibiotics in the 6 months prior to study participation was an exclusion criterion.
Although there were no significant changes in overall bacterial alpha diversity, there were both skin site- and disease status-specific changes. Moreover, bacterial beta diversity showed significant differences for both skin site and lesional versus nonlesional skin using the Bray-Curtis dissimilarity indices ($P < 0.05$). Firmicutes were the most prevalent bacterial phyla identified from psoriatic lesional skin, with reduced prevalence in non-lesional skin and skin from healthy controls. The proportion of Actinobacteria increased from psoriasis lesional to non-lesional to healthy control skin. Genera-level analysis identified Staphylococcus aureus and Propionibacteria as the most prevalent bacteria in psoriasis lesional and healthy control skin respectively. Interestingly, the changes in the cutaneous microbiome composition were supported by data generated from bacterial culture combined with spectrometry.
These data document the existence of significant shifts in composition of the human integumentary bacterial flora associated with both psoriasis and the presence of lesional skin, confirmed using bacterial culture combined with spectrometry. Future studies will address whether the changes are persistent and/or influenced by systemic treatment and whether rapid identification of the cutaneous microbiome may serve as a future individualized biomarker of disease activity.

P268

Laminin Gamma 1 Pemphigoid: a novel immunoblot assay with recombinant Laminin Gamma 1 protein

F. Solimani[1], M. Schaller[2], R. Eming[1], M. Hertl[1] and T. Schmidt[1]
[1]Philipps-University Marburg, Department of Dermatology and Allergology, Marburg, Germany; [2]Universitäts-Hautklinik Tuebingen, Tuebingen, Germany

Laminin Gamma 1 (LNγ1) pemphigoid is a rare subepidermal blistering disease which clinically resembles bullous pemphigoid (BP) and is frequently associated with psoriasis vulgaris. In contrast to BP, IgG autoantibodies from LNγ1 pemphigoid patients react with a 200 kDa protein located in the dermal side of saline-split human skin and has recently been linked to the γ1 chain of laminin. We here describe a novel immunoblot assay which helps identify patients with LNγ1 pemphigoid. A total of six LNγ1 pemphigoid sera showed IgG reactivity with the dermal side of saltsplit skin but did not react with autoantigens of the dermal-epidermal basement membrane zone such as BP180, BP230, collagen VII and LN-332. All the sera were then subjected to immunoblot analysis with three recombinant proteins, i.e. LN-421, LN-111, and the single LNγ1 chain. Only 3/6 of the LNγ1 pemphigoid sera reacted with LN-111 but all of 6 sera reacted with LN-421 and the single LNγ1 chain. In contrast, none of the studied BP sera ($n = 10$) and sera from patients with psoriasis ($n = 10$) showed IgG reactivity against these LNγ1 recombinants. Our findings strongly suggest that the established immunoblot assay with recombinant LNγ1 proteins is very helpful in establishing LNγ1 pemphigoid which is often misdiagnosed based on the findings of indirect immunofluorescence.

P269

ABCB5 is a stem cell cycle regulator in MSCs of the skin

J. de Vries[1], B. Meier[2], S. Vander Beken[3], D. Jiang[1], N. Y. Frank[4], P. Kampilafkos[5], A. Kluth[6], C. Ganss[6], M. H. Frank[5,4] and K. Scharffetter-Kochanek[1]
[1]University of Ulm, Department of Dermatology and Allergic Diseases, 89081 Ulm, Germany; [2]University of Zürich, Department of Dermatology, Zurich, Switzerland; [3]Bredent Medical GmbH & Co.KG, 89250 Senden, Germany; [4]Brigham and Women's Hospital, Transplantation Research Center, Boston, USA; [5]Brigham and Women's Hospital, Harvard Medical School, Harvard Skin Disease Research Center, Department of Dermatology, Boston, USA; [6]TICEBA Lifescience GmbH, 89120 Heidelberg, Germany

The ATP-binding cassette transporter ABCB5 was originally found to be expressed on epidermal CD133+ malignant melanoma initiating stem cells and in several other malignancies, responsible for increased resistance against a wide spectrum of chemotherapeutic drugs. We here describe a novel population of dermal ABCB5+ multipotent stromal cells (MSCs) with a surface marker expression profile similar to conventional MSCs and immunomodulatory properties in macrophage activation distinct from dermal fibroblasts. ABCB5+ MSCs displayed self-renewal potency as assessed by means of a self-renewal assay *in vitro*. In addition, a tripotent differentiation capacity into adipogenic, osteogenic and cartilage lineages was observed on a single cell-derived clonal level. ABCB5+ MSCs co-expressed the stem cell marker SSEA-4 *in vivo* and *in vitro* as well as the stem cell-associated transcription factor Sox2 *in vitro*. We here uncovered a functional role of ABCB5 in cell cycle regulation of ABCB5+ MSCs. Blocking the ABCB5 function by a neutralizing anti-ABCB5 antibody resulted in an increase in actively proliferating ABCB5+ cells, while isotype matched control antibodies did not affect MSC proliferation. As opposed to high proliferation in ABCB5+ MSCs, neutralizing anti- ABCB5 antibody did neither affect proliferation rates of ABCB5- fibroblasts nor that of ABCB5-adipose tissue-derived MSCs. Of note, an age-dependent significant decrease in ABCB5+ MSC numbers from 3.2% of total dermal cells in the skin of young individuals to only 1.6% of total dermal cells in the skin of individuals above 70 years of age was found by immunostaining. Double staining for ABCB5 and the endothelial cell marker CD31 identified ABCB5+ MSCs as a population distinct from endothelial cells, residing in a perivascular and interfollicular localisation. Notably, an age-dependent change in niche preference of ABCB5+ MSCs was observed. While 75% of ABCB5+ MSCs reside in close association to endothelial cells of small vessels in the skin of young individuals, only 10% of ABCB5+ MSCs were found in a perivascular localization, but rather in an interfollicular localisation in the skin of old individuals. Double staining for ABCB5 and NG2, an established pericyte marker, showed ABCB5+ MSCs to be distinct from pericytes. To further characterize the endogenous niche of ABCB5+ MSCS in the skin, double stainings of ABCB5, NG2 and the extracellular matrix component osteopontin were performed, respectively. Interestingly, in parallel to the decrease in perivascular niche preference of ABCB5+ MSCs, also the number of dermal pericytes co-expressing NG2 and osteopontin significantly decreased in the skin of old compared to young individuals. These data provide several lines of evidence that ABCB5+ MSCs constitute a novel mesenchymal stem cell population in human skin which – due to its capacity to control quiescence and self-renewal – most likely maintain tissue homeostasis and regeneration, and – if decreased in numbers with reduced replenishment of cell loss – may even promote skin aging.

P270

FLG mutations lead to an inflammatory phenotype in AD HEEs

S. Blunder[1], V. Moosbrugger-Martinz[1], R. Rühl[2], A. Geisler[1], C. Krimmel[1], R. Gruber[1], M. Schmuth[1] and S. Dubrac[1]
[1]Innsbruck Medical University, Dermatology, Innsbruck, Austria; [2]University of Debrecen, Debrecen, Hungary

Loss-of-function mutations in filaggrin (FLG) cause Ichthyosis Vulgaris (IV) and represent the major predisposing risk factor in atopic dermatitis (AD). While both conditions are characterized by epidermal barrier impairment, only AD exhibits signs of cutaneous inflammation. Previous reports utilized organotypic skin cultures knocked-down for FLG or treated with Th-2 cytokines as models for IV and AD.
This work aims at delineating the role of FLG loss-of-function mutations in IV and AD using human epidermal equivalents (HEEs) generated with keratinocytes isolated from non-lesional skin of FLG WT AD (WT/WT), FLG mutated AD (FLG/WT), IV (FLG/FLG) and healthy donors. Morphological analyses show that keratohyalin granules are absent in IV (FLG/FLG) HEEs. Barrier permeability assays demonstrate no detectable impaired barrier function in all HEEs. Gene expression analyses show an increase of TNFα and TARC in AD (FLG/WT) HEEs and of IL-1β in both AD (WT/WT) and AD (FLG/WT) HEEs. FLG is decreased in AD (FLG/WT) HEEs, whereas protein levels are reduced. Hornerin mRNA levels are increased in AD (WT/WT) and AD (FLG/WT) HEEs, but reduced at protein level. Loricrin remained unchanged at the mRNA and protein level. Lipidomic analyses show increased levels of arachidonic acid (AA) and 12-LOX pathway metabolites in AD (FLG/WT) HEEs as compared to WT controls. AA treatment of ctrl HEEs increases IL-1β and TARC expression. Converseley, treatment of ctrl HEEs with 12-HETE leads to decreased FLG, LOR and HRNR mRNA levels.
These data demonstrate for the first time that HEEs generated from non-lesional AD (WT/WT), AD (FLG/WT) and IV (FLG/FLG) keratinocytes share common features with AD and IV skin. Furthermore, these results evidence that FLG mutations worsen inflammation in AD by triggering expression of inflammatory cytokines and lipids.

P271

Does PPARγ-mediated signalling regulate mitochondrial energy metabolism human hair follicle epithelium?

M. Alam[1], Y. Ramot[2], L. Ponce[1], J. Chéret[1,3], M. Bertolini[1,3] and R. Paus[4]
[1]University of Muenster, Dermatology, 48149 Muenster, Germany; [2]Hadassah-Hebrew University Medical Center, Dermatology, Jerusalem, Israel; [3]Monasterium Laboratory, Skin & Hair Research, 48149 Muenster, Germany; [4]University of Manchester, Dermatology, Manchester, UK

There is increasing interest in Peroxisome proliferator-activated receptors (PPARs) in human skin biology and pathology, as they are prominently expressed in human skin and its appendages. Recently, PPARγ mediated signalling has become appreciated as a key regulator of human epithelial stem cell functions and has also been implicated in the regulation of mitochondrial energy metabolism. In human HF biology, PPARγ-mediated signalling may exert protective effects for epithelial stem cells, while agonistic PPARγ modulators can inhibit hair growth by inducing catagen and inhibiting matrix keratinocyte proliferation. PPARγ stimulation has been shown to enhance mitochondrial function, e.g. in adipose and muscle tissue and the brain. However, it remains unknown whether PPARγ-stimulation impacts on intrafollicular mitochondrial function, a key factor in HF energy metabolism. We have probed this hypothesis by stimulating microdissected, organ-cultured human scalp HFs with GMG-43AC (0.01–1 mM), a selective PPARγ modulator. Preliminary microarray analysis results suggested that this PPARg modulator may regulate the intrafollicular transcription of several genes involved in the control of mitochondrial function. Indeed, qRT-PCR revealed increased transcription of key genes of mitochondrial biology (PGC1α, MTCO1, TFAM and SLC25A3). GMG-43AC significantly increased immunoreactivity for MTCO1, a key enzyme in the respiratory chain, for TFAM, a key transcription factor for mtDNA synthesis and porin, a marker for mitochondria mass, in the proximal outer root sheath cells and hair matrix keratinocytes of human scalp hair follicles *in situ*. These pilot observations suggest that PPARγ-mediated signalling is a novel, therapeutically targetable player in the energy metabolism of human scalp HFs in health and disease.

P272

CD73 and CD39: immune-inhibitory ecto-enzymes as novel regulators of human hair growth

M. Bertolini[1,2], L. Ponce[1], K. Figlak[1], Y. Uchida[1,3], J. Chéret[1,2], H. Waldmann[4] and R. Paus[1,5]
[1]University of Muenster, Dermatology, Muenster, Germany; [2]Monasterium Laboratory, Muenster, Germany; [3]Kagoshima University, Dermatology, Kagoshima, Japan; [4]Oxford University, Oxford, UK; [5]University of Manchester, Centre for Dermatology Research, Institute of Inflammation and Repair, Manchester, UK

The immuno-inhibitory surface ecto-enzymes, CD39 (ectonucleoside triphosphate diphosphohydrolase 1) and CD73 (ecto-5′-nucleotidase), are responsible for dephosphorylating ATP to AMP or for converting AMP into adenosine, respectively, and are recognized for their role in maintaining immune privilege (IP). Given that adenosine has previously been reported to promote hair growth in mouse vibrissae and human scalp hair follicles (HFs) and that HFs enjoy IP, we have investigated here for the first time whether these ecto-enzymes play any role in hair biology. First, we have characterized the expression of CD39 and CD73 in human scalp HFs. CD73 and CD39 protein are strongly expressed in the HF mesenchyme, i.e. connective tissue sheath (CTS) and in the dermal papilla of anagen VI HFs, namely by endothelial and immune cells. Interestingly, CD73 protein and mRNA (*in situ* hybridization) are also expressed in the HF epithelium, specifically in the inner root sheath (IRS), where CD73 co-localizes with CK74, including prominent CD73 expression by the mysterious 'Fluegel-Zellen' (winged cells) of the IRS′s Huxley layer. Yet, neither of these locations correlates with classical sites of intrafollicuar IP. Since CD73 and CD39 transcription increases significantly during apoptosisdriven catagen development, we investigated, next, whether the CD73 specific antagonist, adenosine 5′-(α,β-methylene)diphosphate (APCP), impacts on human hair growth *ex vivo*. Indeed, APCP treatment strongly inhibited hair shaft production *ex vivo* and induced premature catagen development. Since CTS immunocytes (mast cells, macrophages) regulate murine HF cycling, it remains as yet unclear whether premature catagen induction by CD73 inhibition reflects a direct effect on the HF epithelium or is mediated via the HF mesenchyme. When we attempted to knock-down CD73 in organ-cultured human scalp HFs, CD73 mRNA was strongly down-regulated after 6 h of CD73 siRNA treatment. Yet, CD73 *in situ* protein expression was only decreased in the HF mesenchyme, but not in the IRS, 5 days after CD73 silencing in CD73 siRNA treated compared to control HFs, and no difference in hair shaft growth or catagen induction was seen. This could suggest that CD73 activity in the HF epithelium is important for hair cycle regulation rather than that in the HF mesenchyme and its immunocytes. We are currently exploring whether CD73 inhibition-dependent catagen induction is really due to a decrease of intrafollicular adenosine production or reflects other mechanisms, and how CD73 inhibition impacts on the human HF immune system. In summary, the current pilot study introduces CD73 and CD39 as new enzymatic players in hair biology and suggest that the therapeutic manipulation of intrafollicular ecto-enzyme activity, namely that associated with CD73, is a plausible novel strategy for managing human hair growth disorders.

P273

The presence of peptidergic myelinated sensory nerve fibers in re-innervated human skin model promotes mast cells survival and may induce their maturation from resident progenitor cells

J. Chéret[1,2], L. Ponce[1], C. Le Gall-Ianotto[3], L. Misery[3], M. Bertolini[1,2] and R. Paus[1,4] *[1]University of Muenster, 48149 Muenster, Germany; [2]Monasterium Laboratory, 48149 Muenster, Germany; [3]University of Brest, 29200 Brest, France; [4]University of Mancherster, M13 9PL Manchester, UK*

Peptidergic nerve fibers (NFs) innervate various mammalian tissues, including the skin. Those NFs are found to be in close contact with mast cells (MCs) and their interaction allows bidirectional cooperation. In fact, peptidergic NFs release substance P, calcitonin gene-related peptide (CGRP) and vasoactive intestinal peptide (VIP) that can activate mast cells by stimulating specific surface MC receptors. At the same time, MCs can release nerve growth factor (NGF), a fundamental neuromediator required for nerve survival.

In addition, it has been previously shown in mouse skin that close contacts between MCs and NFs induce MC maturation. However, it is still unknown whether this phenomenon also occurs in human skin. To investigate this, we have employed a sensory re-innervated human skin organ culture model in which healthy adult human scalp skin is co-cultured with primary sensory neurons from rat dorsal root ganglion (DRG) in serum-free medium. The co-culture has been maintained *ex vivo* for 17 days. Re-innervated and non-innervated (control skin, skin cultured in the absence of primary sensory neurons from rat DRG) skin samples were harvested and studied at day 5, 11 and 17 of culture.

As expected, in non-innervated control skin, no human or rat NFs (assessed by pgp9.5 and rat myelin basic protein (MBP) immunostainings) could be detected in all time points. Interestingly, in re-innervated skin, the number of rat NFs increased gradually during the co-culture. The newly generated rat NFs firstly surrounded the hair follicles and then the vicinity of dermal MCs. Epidermis was also fully reinnervated by day 11. Importantly, not only non-myelinated (ratMBP-pgp9.5+) but also myelinated NFs (mNFs; ratMBP+pgp9.5+) re-innervated the human skin during the co-culture, the latter being preferentially in contact with both c-Kit+ or Tryptase+ MCs.

During the culture at all time points, c-Kit+ and Tryptase+ MC number was significantly increased in re-innervated skin compared to control. This suggests that the presence of a functional nervous system in the skin promotes the survival of both immature and mature MCs. However, MC degranulation was largely unaffected. In addition, the number and the percentage of c-Kit/Tryptase double-positive cells further increased during the culture in re-innervated skin, suggesting that the presence of a functional nervous system may lead to the maturation of MCs from MC progenitors *in situ*. This is further supported by the fact that MC proliferation, assessed by Ki-67/c-Kit double-staining, was largely unaffected in re-innervated skin.

Furthermore, in re-innervated skin, rat mNFs build a 'cage-like structure' around the c-Kit+ or Tryptase+ MCs which could be visualized at all time points during the coculture. As previously suggested for mouse skin MCs, these particular enclosed MCs may serve as sentinel in the skin, important for initiating the neurogenic inflammation process by activating the nerve endings.

In order to further dissect this hypothesis, we have investigated the expression profile of different neuromediators (CGRP, VIP and NGF) in rat NFs, Indeed, these neuromediators are all released by rat mNFs. Moreover, the high affinity NGF receptor (TrKA) is expressed by the rat NFs and neighbouring MCs. The presence of CGRP and VIP receptors (CGRPR and VPACs, respectively) is also currently being investigated.

Taken together, these findings suggest that, as well as in mice *in vivo*, the sensory re-innervation of human skin promotes skin MC survival and possibly maturation from MC progenitors.

P274

Severe alterations of body image in patients suffering from Acne inversa

A. Kurek[1], E. Peters[2,3], R. Sabat[1,4], W. Sterry[2] and S. Schneider-Burrus[2] *[1]University Hospital Charité, Psoriasis Research and Treatment Center, Dermatology/Medical Immunology, Berlin, Germany; [2]University Hospital Charité, Department of Dermatology and Allergy, Berlin, Germany; [3]University Hospital Charité, Berlin, and Justus-Liebig University, Psychoneuroimmunology, Departments of Psychosomatics and Psychotherapy, Gießen, Germany; [4]University Hospital Charité, Research Center Immunosciences, Berlin, Germany*

Background: Hidradenitis suppurativa (HS) leads chronically to disfigurement and painful eruptions in mainly intimate areas. We hypothesized body image dissatisfaction in HS patients.

Objectives: We studied body image in patients with HS and control subjects. Additionally, we evaluated whether disease severity and co-existing conditions (obesity, depression and anxiety) are linked to the body image and its subscales.

Methods: The Frankfurt Body Concept Scale (FKKS) and the Hospital Anxiety and Depression Scale (HADS) were given to 44 HS-patients and 43 controls to assess body image in HS-patients and in age-, gender- and BMI-matched controls. Moreover, correlation between the scales of FKKS, HADS and disease features was calculated.

Results: This study demonstrated for the first time that HS has a profound impact on body image (FKKS sum score 234.2 $\pm$ 5.4 in patients and 276.9 $\pm$ 5.7 in controls, $P < 0.001$). There was a strong correlation between the extent of body image disruption and BMI ($P < 0.001$), HADS depression score ($P < 0.001$) and HADS anxiety score ($P = 0.034$). No association was found between the FKKS score and the severity of HS-disease ($P = 0.146$), age at onset of disease ($P = 0.577$) and duration of disease (0.288).

Limitations: Small sample size is the main limitation of this study.

Conclusions: Patients with HS suffer from major body image impairments independent of the extent of the disease. This body image impairment might lead to depression and anxiety, disorders that have been largely acknowledged in HSpatients.

P275

Pilot study for the investigation of three dimensional human skin equivalents by 5D intravital multiphoton tomography

F. Valentin[1], C. Mess[1], I. Hausser[2], V. Huck[3] and V. Oji[1] *[1]University of Muenster, Department of Dermatology, Muenster, Germany; [2]University Hospital Heidelberg, Institute of Pathology, Heidelberg, Germany; [3]University Hospital Mannheim, Department of Dermatology, Venereology and Allergology, Mannheim, Germany*

5D intravital multiphoton tomography (5D-IVT) is a powerful tool for non-invasive examination of human skin. The technique provides horizontal images and offers the possibility of stacking single fluorescence pictures to create a three dimensional multilayer model of the skin. In contrast to classical investigation methods such as skin histology or electron microscopy 5D-IVT can be performed *in vivo* and is not based on a skin biopsy procedure.

The present study for the first time demonstrates that 5D-IVT is also applicable for the analysis of three dimensional human (3D) skin equivalents. Fluorescence lifetime imaging microscopy (FLIM) was applied detecting different fluorescence lifetimes of free and protein-bound NADH. Skin equivalents were analyzed with an excitation wavelength of 715 nm. The resultant fluorescence signals of cell metabolism enabled a clear discrimination of the different skin layers. Thereby, images of 3D skin equivalents correlated with those of human skin.

Hence, this pilot study shows that 5D-IVT may provide a novel technique for *in vitro* investigations of human skin. Moreover, fluorescence lifetime imaging can be used to analyze and visualize cell activity within the different layers of the skin model.

Author index

F

G

H

I

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

Subject index