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PO01

Desensitizing latex sensitizations with recombinant latex allergens – the ImmunoSolid phase allergen chip (ISAC®)

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Latex allergens can be diagnosed by using different test methods such as IgG quantification, Western Blot (WB), CAST (cellular antigen stimulation test) and in vivo methods (skin prick test (SPT), gel use test). A sophisticated method is the measurement of specific IgE against single allergens found in natural latex. These allergens are commercially available as recombinant allergens. Phadiatop® (Upjohn, Sweden) provides two methods for detection of specific IgE against recombinant latex allergens: the conventional Phadiatop® allergen chip with Hev b 5 spiked ImmunoCAP (Phadia, Freiburg, Germany) test system (available recombinant allergens: Hev b 1, Hev b 5, Hev b 6, Hev b 6.01, Hev b 6.02, Hev b 8, Hev b 9, Hev b 11) and the Immuno Solid-phase Allergen Chip (ISAC®, Genomone Diagnostics, Freiburg, Germany), which enables measurement of specific IgE against 15 different allergens from diverse sources including five latex allergens (Hev b 1, Hev b 3, Hev b 5 and Hev b 6).

The aim of the study was to compare the diagnostic sensitivity and specificity of the ISAC® test and the conventional Hev b 5 spiked ImmunoCAP® latex extract. Only 22 of 40 subjects with known hand eczema and positive latex SPT, WB and CAST showed sensitization against at least one latex allergen on the ISAC® (sensitivity ISAC® 59%, specificity ImmunoCAP® latex extract 99%). The most sensitized patients were of the occupational group. One of these patients showed the same allergens against Hev b 5 which is not provided on the ISAC® allergy chip.

Although the ISAC® test seems to be more sensitive in detecting latex sensitization, the ISAC® and CAST results are not always in line, as CAST results are known to reflect IgM, whereas the ISAC® test results are IgE. In conclusion, the ISAC® test seems to be a promising and non-invasive tool for the diagnosis of latex sensitization.

PO02

Establishment of an IgE- and mast cell (histamine and platelet-activating factor) dependent humanized mouse model for allergic gut inflammation

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Development of humanized mouse models is of great interest to study allergic diseases and their treatment.

Here we report the establishment of a new mouse model system. In the study we developed a humanized mouse model of allergic gut inflammation and analyzed the underlying immunological mechanisms. NOD-scid/c-/- mice were immunotolerized with human PBMC from grass or birch pollen allergic donors together with the respective allergens. After 10 weeks some were collected for detection of human total and allergen-specific IgE. Then, mice were challenged with the allergen rectally and gut inflammation was monitored histologically and by a high resolution videomicroscopy.

This model allows a better understanding of human allergic asthma as well as allergic diseases of the gastrointestinal tract and each intestinal layer. The mouse model of TNBS-induced colitis is pathophysiologically similar to Crohn’s disease and hence, is suitable for the investigation of cellular mechanisms. In TNBS-induced colitis, mice develop a persistent colitis which is mediated by CD4+ T helper 1 T cells. Previously, we demonstrated that specific oral application of different allergens which prevents the development of a contact hypersensitivity reaction (CHS), a CD4+ Tc-mediated reaction. After single application of TNBS-induced colitis, mice develop a persistent colitis which is mediated by CD4+ Tc helper 1 T cells. In our study, we demonstrated that independent of the site of tolerance induction CD4+ Tc-mediated skin inflammation is as well as as CD4+ Tc helper 1 T cell-mediated colitis.

PO03

Glutaraldehyde modified allergoids induce diminished T cell responses

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Allergic-specific immunotherapy (IT) is a clinically effective therapy for IgE mediated allergic diseases. In an attempt to reduce the risk of IgE-mediated side effects, chemically modified allergoids have been introduced to reduce IgG-binding and retain or increase T cell activation.

The aim of the study was to analyse the different responses of murine T cells after stimulation with allergoids, concerning allergen uptake, T cell and basophil activation.

Therefore, we investigated the monocyte-derived immature dendritic cells (iDC) with Phelgm proteins or beta1,2-macroglobulin extracts or with the corresponding allergoids, modified with glutaraldehyde or formaldehyde. In order to mimic matured immune dendritic cells we co-cultured with autologous CD4+ T cells. Allergoid exposure was tested by basophil activation assay (leakage/ c-kit release).

In addition the uptake of intact allergens and allergoids by immature DC was analysed. The proliferation and IL-12, IL-10, IL-13 and IFN-gamma production of glutaraldehyde allergoid-stimulated CD4+ T cells were reduced compared to intact allergens, and formaldehyde-allergoid-stimulated CD4+ T cells. In line with this, glutaraldehyde modified allergoids were internalized more slowly. Allergoids modified with glutaraldehyde also showed a decreased leakage/ release.

These findings suggest that B cell epitopes of modified allergoids were destroyed most efficiently by reaction with glutaraldehyde. Glutaraldehyde modified allergoids, investigated in this study, seemed to retain both B cell and T cell responses. Biochemical and immunological differences among allergoids may result from differences in modification and aggregation.

PO04

Epicutaneously- and orally-induced tolerance to allergens protects from allergic reactions to alpha-GAL


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Allergic reactions to alpha-galactose (alpha-GAL) have been a significant emerging problem in clinical practice. A sensitive and specific diagnostic method to detect IgE to alpha-GAL is important to identify patients at risk for IgE-mediated adverse reactions to alpha-GAL containing foods. In previous studies, we were able to demonstrate that oral alpha-GAL application leads to Th2 cytokine production (IFN-gamma-IL-4) and that the intestinal mucosa is involved in IgE binding to alpha-GAL. Hence, we hypothesize that induction of alpha-GAL-specific IgE as well as allergen-specific proliferation and cytokine production after restimulation with alpha-GAL can be used as marker for alpha-GAL-induced tolerance.

A patient with proven Cetuximab allergy served as positive control.

The proliferation and IL-4, IL-10, IL-13 and IFN-gamma production of glutaraldehyde allergoid-stimulated CD4+ T cells was analysed. After cytokine induced maturation the antigen pulsed mature dendritic cells were co-cultured with T cells from patients with known alpha-GAL allergy and from healthy controls. The proliferation and cytokine production of glutaraldehyde-allergoid-stimulated CD4+ T cells was significantly reduced in alpha-GAL allergic patients compared to healthy controls.

In conclusion, alpha-GAL-specific IgE allergy is a significant emerging problem in clinical practice. Our results strongly suggest that induction of alpha-GAL-specific IgE as well as allergen-specific proliferation and cytokine production after restimulation with alpha-GAL can be used as marker for alpha-GAL-induced tolerance.

PO05

Interplay between CD4+CD25+ regulatory T cells, tolerogenic CD11c+ dendritic cells and CD8+ suppressor T cells is critical for tolerance to contact allergens


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Low-zone tolerance (LZT) to contact allergens might be a natural mechanism for the regulation and circumvention of allergen sensitization in humans. LZT is induced by epicutaneous applications of subimmunogenic doses of hapten (TNBS) before sensitization and affects the course of the TNRs-induced colitis mimicking Crohn’s disease in humans. Notably, it was found that the application of repeated oral and epicutaneous treatments with low doses of allergens on the outcome of the TNBS-colon mimicking Crohn’s disease in humans. Notably, it was found that the application of repeated oral and epicutaneous treatments with low doses of allergens on the outcome of the TNBS-colon mimicking Crohn’s disease in humans.

The aim of this study was to compare the effectiveness of oral and epicutaneous treatments with low dose of allergens on the outcome of the TNBS-colon mimicking Crohn’s disease in humans. Notably, it was found that the application of repeated oral and epicutaneous treatments with low doses of allergens on the outcome of the TNBS-colon mimicking Crohn’s disease in humans. Notably, it was found that the application of repeated oral and epicutaneous treatments with low doses of allergens on the outcome of the TNBS-colon mimicking Crohn’s disease in humans.
Penetration of topicaly applied pollen allergens into Langenhan's cells

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Background: It is well known that topicaly applied pollen allergens (PA) can deteriorate skin condi-
tion in sensitized atopic patients. Only recently, in this context hair follicles have been discussed to ele-
brate an allergenic role. They are surrounded by a dense network of Langerhans cells (LC). Investiga-
tions have shown that the penetration of pollen allergens into the hair follicles was significantly re-
duced when they had been treated with specific medical skin care products. But up to now there is no
direct evidence that the PA can cross the skin barrier into the living cells.

Objectives: In the present study, the uptake of topicaly applied PA into LC on excised human skin was
investigated on excised human skin after barrier disruption in order to imitate atopic skin condition. A
PA-uptake was compared in skin specimens with and without treatment with a medical skin care product.
As a control the uptake of PA in LC on intact skin was investigated.

Methods: Grass pollen allergens were labelled with a fluorescent dye and applied onto excised human
skin where the skin barrier was disrupted by cryostat surface scraping. Part of the samples was pre-
incubated with Eucon-PHFS Lottum F. Epidermal suspension cells were generated and separa-
tion of undamagedLCLs was performed. The uptake of PA into LCs was measured using laser
scanning microscopy. PA were applied on undamaged skin as control.

Results: Compared to skin, pollen allergens could be detected in about 80% of the LC after application
on barrier disrupted skin. The pretreatment with the skin care product reduced the penetration of PA of
about 10%.

Conclusion: It could be demonstrated that PA penetrate into the viable skin and reach the LC when the
skin barrier is disrupted. A higher barrier enhancing formulation reduced the penetration of the PA into
the LC significantly. For helatonic treatment of the type I allergy, greater atten-
tion should be paid to the development of a specific skin barrier enhancing formulation that could pro-
vide an additional allergy prevention strategy.

A novel method for flow cytometry-based analysis of T cell cross- reactivity of birch pollen allergens Bet v 1 and homologous food allergens

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Background: Birch pollen allergy is one of the most prevalent allergic diseases in northern Europe. Among birch-poll-
en allergic patients approximately 70% experience secondary food allergy e.g. to hazelnuts, carrots, apples or others. Evidence has shown that this oral allergy syndrome (OAS) is mediated by IgE and T cell cross-reactivity to proteins of the related protein family (F/R-B) which are homologous to Bet v 1 (e.g. cor 1 0401, Dan v 1 in cor1). In our former studies, the T cell response to recombinant Bet v 1 and Cor 1 0401 in primary and secondary stimulation induced by human mature monocytes was determined. Notably we found very little cross reactivity in response to these allergens using the parameter of T cell proliferation in 3H-Thymidine assays. However, this method entails the additional problem that it does not allow for discrimination between cells proliferating after secondary stimulation only and those proliferating after primary and secondary stim-
ulation. To overcome this problem we have established a flow cytometry-based method for the analysis of T cell cross reactivity in birch pollen-allergic patients with secondary sensitization. For this purpose, we simultaneously performed a flow cytometry-based method for the analysis of T cell cross reactivity on one single cell level.

A new method for flow cytometry-based analysis of T cell cross reactivity of birch pollen allergens Bet v 1 and homologous food allergens

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ulation. To overcome this problem we have established a flow cytometry-based method for the analysis of T cell cross reactivity in birch pollen-allergic patients with secondary sensitization. For this purpose, we simultaneously performed a flow cytometry-based method for the analysis of T cell cross reactivity on one single cell level.
P014 (V36) MN8001, a dendritic polyglycerol, diminishes allergic type I and IV reactions in mice.

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Introduction: MN8001 is the most frequent cause of contact hypersensitivity (CHS) in industrialized countries. Both a lymphocyte-specific signal and a proinflammatory signal are required for efficient CHS. Therefore, we analyzed whether MN8001 can inhibit MC degranulation in PSA, we measured mouse MC protease-1 (mMCP-2) as a marker for neutrophil influx and eosinophil infiltration, as well as the proinflammatory mediator TNF-α. Our results indicate that MN8001-treated mice showed a significant decrease in TNF-α and mMCP-2 levels, indicating that MN8001 has an inhibitory effect on the allergic reaction in vivo and is a promising candidate for the treatment of CHS.

P015 Allergen-specific blocking activity of IgG antibodies induced by specific immunotherapy.

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Introduction: Specific immunotherapy (SIT) is the standard treatment for allergic diseases. However, it is known that SIT can cause adverse events, such as anaphylaxis, which limits its use. Therefore, we investigated the allergen-specific IgG antibodies induced by SIT and their blocking activity.

Methods: SIT-naive patients were immunized with increasing doses of the allergen for 1 year. Serum samples were collected before and after the completion of SIT. The allergen-specific IgG antibodies were determined by ELISA and their blocking activity was measured in an in vitro assay.

Results: After the completion of SIT, the allergen-specific IgG antibodies showed a significant increase compared to baseline. Moreover, the allergen-specific IgG antibodies were able to block the allergen-specific T cell proliferation in vitro.

Conclusion: The allergen-specific IgG antibodies induced by SIT have a significant blocking activity and could be a potential target for the development of new immunotherapies.

P016 (V13) Protective role of CB1 receptors on keratinocytes in a mouse model of allergic contact dermatitis.

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Introduction: CB1 receptors are G protein-coupled receptors (GPCRs) that are highly expressed in keratinocytes and play a role in the pathogenesis of allergic contact dermatitis (ACD). We hypothesized that CB1 receptors may have a protective role in ACD and investigated this in a mouse model.

Methods: ACD was induced in C57BL/6 mice by topical application of a hapten (2,4,6-trinitro-1-chlorobenzene). Animals were treated with CB1 receptor agonists or antagonists before and during the induction of ACD.

Results: Pretreatment with a CB1 receptor agonist significantly reduced the clinical severity of ACD. In contrast, treatment with a CB1 receptor antagonist exacerbated ACD.

Conclusion: CB1 receptors play a protective role in allergic contact dermatitis and may be a potential target for the development of new therapies.

P017 Effects of different brands of medical compression stockings (MCS) on skin barrier function, microbial status and wearing comfort.

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Introduction: Medical compression stockings (MCS) are used to improve venous return and prevent edema. However, the effects of different brands of MCS on skin barrier function and microbial status are not well understood.

Methods: Twelve healthy volunteers wore two different stockings (brands A and B) on each leg for 3 months. Skin barrier function was assessed by measuring transepidermal water loss (TEWL) and skin moisture. Microbial status was assessed by culturing skin samples on selective media.

Results: Wearing stockings with added skin care emulsion (CCE) in comparison with controls (CCS) resulted in significantly higher amounts of bacteria on the skin under the stockings.

Conclusion: Different brands of medical compression stockings may influence the skin barrier function and the skin microbiome. Further studies are needed to investigate the effects of different brands of MCS on skin barrier function and microbial status.

P018 Influence of medical compression stockings on skin barrier function and haemodynamics at patients suffering from chronic venous insufficiency (CVI).

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Introduction: Skin redness and itching are most often recorded complaints by patients suffering from CVI. Wearing medical compression stockings (MCS) can improve skin barrier function and reduce itching.

Methods: Twenty patients suffering from CVI (10 men and 10 women) were randomized to either wearing a control garment (CCS) or wearing a garment with a skin care emulsion (CCE). Skin barrier function, skin moisture, and microbial status were assessed before and after wearing the garments.

Results: Wearing stockings with added skin care emulsion (CCE) in comparison with controls (CCS) resulted in a decrease in TEWL and an increase in skin moisture.

Conclusion: Wearing medical compression stockings with added skin care emulsion can improve skin barrier function and reduce itching in patients suffering from CVI.
Dissecting the molecular mechanisms involved in human endogenous skin ageing in both genders

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The development of genomic tools has facilitated deeper insights into the molecular events underlying human ageing. The identification of pathways affected by skin ageing has led to the development of new strategies to possibly enable skin repair. In the current study, we investigated the gender-specific differences in the gene expression of aged skin between healthy males and females. Whole genome transcriptome gene expression patterns were examined in sun-protected skin obtained from European Caucasian young (20 ± 10 (n = 9), respectively) and elderly adults. Within these, 286 genes exhibited increased and 584 decreased expression with age in females and 213 genes were significantly regulated in skin female in skin of males. Differentially expressed genes were further filtered to identify the actual targets of skin ageing independent from gender. Only two genes showed different regulation with increased expression in male and decreased expression in female skin biopsies. In conclusion, induced fibroblasts and the dermal adipocytes could be used for further apoptotic tests in regenerative medicine (cell and tissue replacement) as well as in cosmetic dermatology.

P027
Age-related skin changes on the molecular level

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Ageing is a complex process that also involves the decline or dysfunction of the skin. The corticotropin-releasing hormone (CRH) system, which is involved in inflammatory skin diseases, permits some prolactin-stimulated receptors (PPAR), known to play a critical role in tumourigenesis and metabolism, to be induced to the receptor subtype CRHR2, which enables different CRH expression. Comparative analysis between NFH-2, BJ, HFF-1 and their iPS cells is in progress. In summary, the CRH system is involved in inflammatory skin diseases and has a potential role in skin ageing.

P028
Generation of induced pluripotent stem cells from healthy and patient-derived human skin biopsies as model for in vitro investigation of systemic diseases

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Our patients have previously shown that genes and gene pathways associated with healthy intrinsically aged are markedly common with those associated with systemic diseases. Induced pluripotent stem cells (iPS) offer crucial potential for disease research, drug screening, toxicology and regenerative medicine. However, little is known about the nature and sequence of molecular events accompanying skin ageing, which is regarded as almost a differentiated cell. Such differentiated cells can still not be the origin for considerable amounts of differentiated adipocytes. It has been reported that about 10% of all cutaneous adipocytes are of dermal origin, which are not dependent on gender, and therefore, on the different hormone status. In conclusion, our study provides biomarkers of endogenous skin ageing in both females and males, which are not dependent on gender, and therefore, on the different hormone status.

P029
Biomarkers for Adamantiades-Behet’s disease

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Purpose of the study was to detect serological predictive course-parameters in Adamantiades-Behet’s disease (ABD). Serum/blood of 122 ABD patients inactive/inactive stages and of 75 controls was screened for IL1β, IL6, IL8, ESR and CRP. Serum IL1β showed significantly increased levels (p = 0.0001) in ABD patients compared to controls. Serum IL8 was elevated (p = 0.002) in ABD inactive stages compared to controls. IL1β and IL6 were significantly elevated (p = 0.0001) in ABD inactive stages compared to controls. IL8 levels were increased in the presence of oral aphthae. In summary, IL1β and IL8 are useful predictive parameters in ABD patients, especially in therapeutic trials.

P030
The seboocyte-own corticosterin-releasing hormone system is an amplifier of inflammation

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Activation of the hypothalamic-pituitary-adrenal axis, which is the main adaptive response to chronic systemic stress, requires production of corticosterin-releasing hormone (CRH). CRH has also been detected in peripheral tissues, including the skin. Human sebaceous glands and cultured human S929 sebocytes express CRH, CRH-binding protein (CRH-BP) and CRH receptors (CRH-R1) and CRH-R2, which can be modulated in in vitro experiments. Results comparing CRH treatment on the skin showed that CRH’s expression increased in the presence of oral aphthae. In summary, the CRH system is involved in inflammatory skin diseases and can be used as a model for the investigation of systemic stress.

P031
The role of the chemokine CXCL1 in epithelialization, inflammation and angiogenesis

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K(D)PT resulted in a significant reduction of total Th17 cells and moreover, decreased the secretion of significant reduction of epidermal thickness and furthermore, decreased the levels of pathogenic Th17 RNA level about 70% without major differences between passage number compared to exclusively Atu-a.

**P027**

**Exonuclease-1 exhibits regulation in apoptosis and proliferation under hormone-dependent conditions in human fibroblasts**

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Introduction: Exonuclease (1) EXO1 is a member of the RAD2 family of structure-specific nucleases and has 5′ to 3′ exonuclease as well as RNase H activity. Genetic analysis has identified roles for EXO1 in mismatch repair, replication, recombination, DNA repair and maintenance of telomeres, which play a crucial role in the maintenance of genome integrity. Aging is known to be associated with an increased rate of DNA damage, which is thought to cause genomic instability. In the present study, the influence of hormone treatment on EXO1 expression has been addressed. EXO1 expression was higher in human skin fibroblasts compared to human primary epidermal keratinocytes and the expression was dependent on the time in passage. Further studies have to be done to quantify the effects of the hormone on other inflammatory mediators.

**P028**

**K/DPT, a tripotent related to the κ-melanocytestimulating hormone, efficiently ameliorates ongoing psoriasis in a humanized mouse model**

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The proinflammatory cytokine-derived tripotent κ-MSH as well as its C-terminal tripotent KPV are known to exhibit potent anti-inflammatory and immunomodulatory effects in vitro and in vivo. Recently, K/DPT, a tripotent derived from a synthetic loop of KPV and structurally similar to KPV but characterized by an increased stability and safety was shown to have identical anti-inflammatory and immunoregulatory effects compared to KPV and κMSH. Of note, the anti-inflammatory effects of K/DPT might be mediated by a reduction of nuclear factor κB (NF-κB) activation, a transcription factor which is crucial for the regulation of inflammatory processes. Efficacy of K/DPT has known to exhibit potent anti-inflammatory and immunomodulatory effects and may be a novel therapeutic approach for treatment of psoriasis.

**P029**

**Pregnane X Receptor (PXR) links xenobiotic metabolism to the cutaneous immune response**

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The pregnane X receptor (PXR) is a ligand-activated transcription factor regulating genes central to drug and hormone metabolism. More recently, we have shown that ligand activation of PXR suppresses the T lymphocyte function. We have here that topical applications of PCN, a well-known activator of mouse PXR, ameliorates psoriasis and allergic contact dermatitis in a PXR-dependent manner. Moreover, retinoids, an activator of the PXR in humans, when applied topically improves allergic contact dermatitis in mice in humanized for PXR and for both PCN and CYP3A, an important PXR downstream gene, Retinol down-regulates the expression of CYP and in PXR-humanized keratinoctyes in an anti-gen-specific and receptor-mediated manner. Conversely, PXR deficient mice exhibit exaggerated irritant and allergic contact dermatitis. Increased our welling is associated with inflammatory cytokines mainly consisting of CD4+ T-lymphocytes. Adoptive transfer experiments demonstrate that T-lymphocytes per se are not responsible for the pro-inflammatory phenomena in PXR deficient mice, suggesting synergy with other cell types, potentially keratinocytes. Furthermore, PXR is expressed by lymphocyte-rich infiltrations and basal keratinocytes of human psoriatic skin. In conclusion, PXR links xenobiotic metabolism to the skin immune response.

**P030**

**Vitamin D treatment suppresses the Th17-induced proinflammatory S100 alarmins psoriasin (S100A7) and koebnerisin (S100A15) in psoriasis**


In psoriasis, an important Th17 cytokine, has been implicated in the pathogenesis of this disease. Hence, the potential role of Th17-induced proinflammatory cytokines of the S100 alarmins proteins of the psoriasin and koebnerisin gene family in psoriasis susceptibility is discussed.

**P031**

**SOX10**

SOX10**

SOX10 molecules with cutaneous antinflammatory activity prime skin for inflammation: a mouse model for psoriasis susceptibility

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Psoriasis is genetically linked to small molecules of the human S100A7A15 subfamily encoded within the psoriasis susceptibility locus at chromosome 1q21. Inflammation-prone psoriatic skin is characterized by constitutively elevated levels of SOX10A15A7 and this has been shown to act in a ligand-dependent manner. The SOX10A15A7 expression was time- and dose-dependently regulated by S100A15. Notably, sox10a15a7 is essential for the pro-inflammatory phenotype in PXR deficient mice, suggesting synergy with other cell types, potentially keratinocytes. Antipsoriatic vitamin D analogs further interfered with the SOX10A15A7 induction by suppressing of S100A15A7 and potentiating their expression with Th17 derived cytokines in keratinocytes. In return, proinflammatory cytokines and the levels of SOX10A15A7 were absent in Th17-deficient mice.

**P032**

**Alpha-MSH inhibits TNF-alpha-mediated responses in human melanocytes**

A. Kedek and M. Buham. Department of Dermatology, University of Münster, 48149 Münster, Germany. Suppression of cytokine (SOCS) are genes which negatively regulate cytokine signaling. Several cytokines such as interleukin (IL)-6, IL-10 or tumor necrosis factor alpha (TNF-alpha) are typically induced after UVB irradiation of epidermal cells and potentiate their expression with T cell-derived Th1/Th17 cytokines. In return, pso-
growth factors, growth factor receptors, adhesion molecules and matrix metalloproteases.

**P037**

**Tonic inhibitory effects of endocannabinoids on human skin mast cell cellfunctions in situ by cannabinoid receptor 1 (CB1)-mediated signalling**

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Since many chronic inflammatory and allergic skin disorders are characterized by excessive mast cell (MC) tissue infiltration, it is clinically important to understand the physiological controls that prevent normal human skin MCs from being activated. The endocannabinoid system (ECS) is a natural neurochemical control system that regulates invertebrates and vertebrates several crucial functions by way of the cannabinoid receptor (CB1)-mediated signalling pathway. We have therefore developed a standardized full-thickness human skin wound healing assay that permits the study of skin cells in situ. In this study, we examined the effect of non-psychoactive endocannabinoids on human skin MCs in situ. Using real-time polymerase chain reaction (RT-PCR), mass spectrometry and cell functional assays, we have demonstrated that CB1 mediates tonic inhibitory effects of endocannabinoids on human skin MCs in situ. CB1 antagonists enhance tonic inhibitory effects of non-psychoactive endocannabinoids on human skin MCs in situ. We have therefore developed a novel model system that permits the study of the tonic inhibitory effects of endocannabinoids on human skin MCs in situ. This model system is important for understanding the physiological control of MC tissue infiltration in chronic inflammatory and allergic skin disorders.

**P038**

**Vaspin and psoriasis**


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**P039**

**Prohormone convertases – novel players in melanoma biology**

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Proprotein or prohormone convertases (PCs) are Ca2+ dependent serine proteases which do not process proteins into biologically active peptides. They are synthesized as zymogens and undergo a posttranslational activation step, in which a signal peptide is cleaved off and an active catalytic site is generated. PCs have been implicated in various physiological processes, including hormone biosynthesis, growth factor processing, prohormone processing, and protein modifications. In this study, we investigated the role of prohormone convertases in the biology of melanoma cells. We found that prohormone convertases are involved in the processing of several proprotein substrates, including the prohormone convertase PC2 and PC5. We also observed that prohormone convertases are upregulated in melanoma cells and that their expression is associated with increased cell proliferation and invasion. These findings suggest that prohormone convertases play a role in the biology of melanoma cells and may be potential targets for the development of novel therapeutic strategies.
Although it has previously been reported that some PCs are overexpressed in a number of solid tumors, little information is available on PCs in melanoma. In order to clarify if PCs are involved in the pathogenesis of melanoma we focused here on subtilisin/kexin converts (SK-1-11)proprotein convertase activator site 1 (PC5/6) and PC7. We investigated the expression and regulation of these proprotein convertases and compared them to other proinflammatory genes (Cox-2, MCP-1) in melanoma. We found that in melanoma cells, PC5/6 expression was up-regulated in cell lines and in primary melanoma samples. These results indicate that proprotein convertases, in particular PC5/6 and PC7, may play a role in the pathogenesis of some solid tumors. Recently, it was also reported that proprotein convertases play an important role in the pathogenesis of melanoma. Furthermore, it was shown that proprotein convertase site 1 protease (S1P), PC5/6 and PC7 are involved in the pathogenesis of melanoma. We found that in melanoma cells, PC5/6 expression was up-regulated in cell lines and in primary melanoma samples. These results indicate that proprotein convertases, in particular PC5/6 and PC7, may play a role in the pathogenesis of some solid tumors.

Expression of the IL-17 pathway in skin lesions of patients with hidradenitis suppurativa (acne inversa)

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Introduction: Hidradenitis suppurativa (acne inversa, HS) has been recently proposed to be classified in the "auto-inflammatory diseases", a group of recurrent, non-infectious inflammatory disorders, with typical absence of pathogens, autoantibodies or antigen-specific T cells. Interleukin (IL)-17, the signature cytokine of Th17 cells, has been proved to play a role in the pathogenesis of rheumatoid arthritis and Crohn's disease, disorders, which belong to "auto inflammatory diseases" and present a high comorbidity with HS. Moreover, IL-17 is the founding member of a group of cytokines initiating the IL-17 pathway and including IL-17 receptor, intercellular adhesion molecule-1, IL-23 and IL-23 receptor. Other cytokines, which are also associated with the IL-17 pathway, are TNF-a and IL-12, a cytokine whose expression is rapidly induced by TNF and IL-12, a cytokine whose expression is rapidly induced by TNF-a and IL-12 and repressed by the TG-β pathway. To corroborate the involvement of Th17 cell cytokines as HS we investigated the IL-17 pathway in skin lesions of HS patients.

Objective: To investigate the expression of the IL-17 pathway in skin lesions of HS patients.

Methods: Gene array and immunohistochemistry experiments were performed to detect the expression of cytokines involved in the IL-17 pathway. The results were compared to those of non-lesional skin from HS patients and of matched (same localization) skin specimens from healthy individuals. Moreover immunohistochemistry experiments of patients with Crohn's disease were used as control.

Results: The gene array experiments detected a significantly higher IL17 expression in lesional skin compared to non-lesional skin of HS patients. Moreover, down-regulation of TNFR2 was found in non-lesional skin of female HS patients compared to healthy female individuals as well as lesional expression of POU in keratinocytes compared to non-lesional. Immunohistochemical staining showed expression of FOXP3 in the perivascular dermal tissue of lesional skin of HS patients whereas TH17-molecules, including IL17A and IL23A, were only found in skin lesions of HS patients. Moreover, down-regulation of TNFAIP3 was found in HS patients compared to those of non-lesional skin from HS patients.
Expression of Lympho-epithelial Kazal-type inhibitor (LEKT)-2 in cutaneous squamous cell carcinoma

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Abstract

Background: To study the role of LEKT-2 in cutaneous squamous cell carcinoma (cSCC). Methods: We performed immunohistochemical analysis of constitutive LEKT-2 expression in normal skin in comparison to epidermal keratinocytes (NHEK), a keratinocyte cell line (HaCaT) and tumor cell lines (SCC12, SCC4, SCC6). Results: LEKT-2 was strongly expressed in normal skin which is constricted to individual keratinocytes in the basal layer. Skin samples of patients with actinic keratosis showed a predominant staining of nearly all keratinocytes in the basal and suprabasal layer. Furthermore we observed a very strong overexpression of LEKT in tissue of SCC. Conclusions: The further course.

High levels of beta2-adrenoceptors in infantile capillary hemangiomas

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Abstract

Introduction: We have recently shown that the non-selective beta-blocker propranolol strongly inhibits growth of infantile capillary hemangiomas and consecutively is proposed as therapeutic option in children suffering from disfiguring hemangiomas. The aim of this study was to evaluate our hypothesis that beta2-adrenoceptors play an important role in the pathogenesis of these lesions.

Results: Infantile capillary hemangiomas express high levels of beta2-adrenoceptors which might mediate their strong responsiveness to the beta-blocker propranolol.

Contact sensitization in the anal and genital area

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Abstract

Background: To evaluate the role of contact sensitization in the anal and genital area of patients with atopic dermatitis (AD), chronic venous insufficiency (CVI) and healthy controls (HC).

Methods: We selectively enrolled patients with AD (n = 114), CVI (n = 38) and HC (n = 87). Patients were sensitized to 12, 18 and 24 different allergens, respectively. Allergic contact dermatitis was diagnosed in 409 (29.8%) of the tested patients. Patients with anogenital disease were sensitized mainly to active agents of topical medications, in particular Bucinex® (5.3%). Sensitization pattern and sensitization rates were compared between patients suffering from AD (n = 108) and CVI (n = 36) and healthy controls (n = 84).

Results: LEKT-2 immunoreactivity was detected at site of prominent hyperkeratosis of SCC. The cumulative 5-year incidence of doctor diagnosed asthma was 150/2927 (5.1%, 95% CI 4.4–6.0) and of doctor diagnosed rhinitis 172/3080 (5.6%, 95% CI 4.9–6.4) respectively.

Conclusions: Expression of LEKT-2 is strongly associated with the development of atopic dermatitis and consecutively may play an important role in the pathogenesis of these lesions.
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Background: An important but yet unknown issue in health services research concerns the degree of variation and the reasons for variation in the treatment of atopic eczema (AE) by medical providers in outpatient care.

Methods: Secondary data analysis utilizing an administrative health database from Germany with complete information on outpatient health services utilization and prescription data of 257.347 individuals. The data were collected from 2010 onwards. The data included 20,469 patients with AE who were treated by dermatologists and pediatricians.

Results: The mean (fifth to 95th percentile) proportion of AE-patients treated by dermatologists and pediatricians were 43% and 35%, respectively. Significant association between atopic eczema and attention-deficit/hyperactivity disorder (ADHD) independent from socioeconomic factors, environmental confounders, and comorbidities. Although the epidemiologic evidence is consistent and suggests that AE predisposes the onset of ADHD, the relative importance of the newly described AE/ADHD comorbidity is still not well understood from a public health perspective.

Discussion: The constant increase of CD4-cell-count/lymphocyte count and the development of anti-inflammatory treatment strategies to improve outpatient care of AE through better standardization of treatment strategies. Future qualitative and quantitative research is needed to better understand the reasons for variation in prescribing patterns and to eventually inform targeted health services research interventions.

P052
Update of epidemiologic and clinical data of Adamantiades-Behet's Disease in Germany

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The German Registry of Adamantiades-Behet’s Disease (ARB) is a registry charted, founded 1990 and aiming at the collection and documentation of all patients suffering from AD. The current registry of ARB is based on the data of 380 patients. 50 of male and 330 of female ARB patients, 287 are of German origin (39.6%), 157 of Turkish origin (43.7%), and 12 of Italian (3.1%), 10 of Greek (2.6%) and nine of Lebanese origin (1.2%). Another 23 patients originate from 27 other countries. The frequencies of the clinical manifestations are: oral ulcers 98.3%, skin lesions 74.9%, genital ulcers 65.8%, arthritis 52.1%, ocular manifestations 51.6%, and pain test positive 30.3%. Severe ocular involvement is significantly associated with HL-A-B5 (P < 0.001) and male gender (P < 0.001). Oral ulcers were with 63.9% the most common clinical manifestation followed by genital (36.8%), arthritis (32.1%), skin lesions (30.6%), genital ulcers (26.4%), and arthritis (26.4%). Among skin lesions, papulopustules could be detected in 58.5%, erythema nodosum-type lesions in 37.3%, proctitis/epilepsia in 10.9%, and manifestation of oral manifestations in 3.9% and manifestations of skin lesions in 1.9%. Disease activity calculated as scoring of ADL (median 2005) showed a correlation with female gender (P < 0.001) and oral ulcers (P < 0.001). Disease activity was significantly lower in Turkish patients than in Germans (P < 0.001). COPD was more frequent among patients with severe ocular involvement (P < 0.001). In general, there was a trend of more frequent ocular involvement in Turkish than in German patients (38.6% vs 48.7%, P = 0.044), but there was no statistical difference in the risk of blindness (6.7% vs 4.5%, P = 0.18 vs 0.8). A similar trend could be observed for folliculitis being more frequent in Turkish than in Germans (59.7% vs 52.7%, P = 0.03), while no statistical difference was observed for folliculitis between the first physician consultation and diagnosis of the disease. 86% currently received lymph drainage, 87% compression therapy. Between the first physician consultation and diagnosis of the disease, 86% currently received lymph drainage, 87% compression therapy. Between the first physician consultation and diagnosis of the disease, 86% currently received lymph drainage, 87% compression therapy. 17% had concomitant disease and 17% had concomitant disease. 17% had concomitant disease and 17% had concomitant disease.

Discussion: The constant increase of CD4-cell-count/lymphocyte count and the development of anti-inflammatory treatment strategies to improve outpatient care of AE through better standardization of treatment strategies. Future qualitative and quantitative research is needed to better understand the reasons for variation in prescribing patterns and to eventually inform targeted health services research interventions.

P053
Differences in the distribution of clinical signs between Adamantiades-Behet’s Disease patients of Turkish and German origin in Germany

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Among the 725 patients with Adamantiades-Behet’s Disease reported to the German Registry of Adamantiades-Behet’s Disease until 2010, 517 were of Turkish (43.7%) and 287 of German origin (39.6%). Androtopism was found among patients of Turkish origin (men:women = 1.84:1, P < 0.001) and Turkish patients had more severe ocular involvement (P < 0.001). Frequencies of other clinical signs showed no significant difference between Turks and Germans. Oral aphthae (98.9% vs 98.0%), genital ulcers (65.8% vs 67.1%), arthritis (50.5% vs 51.6%), and skin lesions (53.2% vs 51.5%) were equally distributed. The frequencies of other clinical signs showed no significant difference between Turks and Germans. Oral aphthae (98.9% vs 98.0%), genital ulcers (65.8% vs 67.1%), arthritis (50.5% vs 51.6%), and skin lesions (53.2% vs 51.5%) were equally distributed. Frequency of other clinical signs showed no significant difference between Turks and Germans. Oral aphthae (98.9% vs 98.0%), genital ulcers (65.8% vs 67.1%), arthritis (50.5% vs 51.6%), and skin lesions (53.2% vs 51.5%) were equally distributed. Between the first physician consultation and diagnosis of the disease, 86% currently received lymph drainage, 87% compression therapy. Between the first physician consultation and diagnosis of the disease, 86% currently received lymph drainage, 87% compression therapy. Between the first physician consultation and diagnosis of the disease, 86% currently received lymph drainage, 87% compression therapy.
Objectives: Our study explored the effect of patients’ age on psoriasis patients’ preferences for treatment features. Methods: Participants included patients identified as having moderate to severe psoriasis according to the 2009 classification criteria of the British Society for Dermatology. Participants were recruited from the outpatient dermatology clinics in the Department of Dermatology at the University Hospital Mannheim, Heidelberg University. Both new patients and patients who previously attended the dermatology clinics were eligible for the study. Participants with psoriatic arthritis, but no skin involvement, and patients <18 years of age were excluded. Consent analysis was utilized to measure participants’ stated preferences for attributes of psoriasis treatment options. Available psoriasis treatment modalities were identified through literature review and consultations with clinical experts. The treatments modalities were decomposed into attributes and attribute levels. Treatment attributes included both process (treatment location, frequency, duration, delivery method, and cost for the individual) and outcome (probability of beneficial effect, magnitude of beneficial effect, duration of benefit, probability of side effects, side effect severity, and side effect reversibility) attributes. Using Sawtooth Softwane (www.sawtoothsoftware.com), hypothetical treatments scenarios were created. Participants were asked to repeatedly choose their preferred treatment option among the hypothetical treatment scenarios presented. Multivariate regression analysis was performed to ascertain the effect of patient characteristics, including age, on the relative importance (partworth utilities) measured for each treatment attribute.

Results: The study sample (n = 163, 58.9% male) included 17% between the ages of 18 and 30 years, 54% between the ages of 31 and 49 years, 25% between the ages of 50 and 64 years, and 17% >65 years old (mean age 49.7 years). The mean PASI was 5.38 and mean DLQI was 7.38. Patients’ preferences for treatment attributes were found to vary among the age groups analysed. Younger patients valued the probability of beneficial effect (and outcome attribute) more than older patients (P = 0.0007 and <0.001, respectively). The relative probability of treatment was higher than their younger counterparts (P = 0.012).

Conclusions: Psoriasis is a common, chronic disease with profound effects on individuals’ quality of life. A broad range of treatment options are available. However, the relative importance of various attributes has not been extensively studied. Age was found to affect the relative importance of different treatment attributes. Younger patients place more weight on the probability of beneficial effect, magnitude of beneficial effect, duration of benefit, probability of side effects, side effect severity, and side effect reversibility than their older counterparts. The observed differences in the treatment attributes valued by different age groups may be due to changes in the disease state and its impact on the patients’ quality of life. Understanding the preferences of different age groups may help in the development of more effective treatment plans for this chronic disease.
with bilateral or abnormal mental health were comparably smaller than the group with normal mental health; in the group with mental normal mental health small effects were more likely to become significant than in the other two groups.

P065 A novel homozygous missense mutation in SLURP1 causes Mal de Meleda with an atypical phenotype

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Abstracts
Effects of splice-site mutations, i.e. the number and nature of transcripts and polyadenylation sites and thus of protein isoforms. Genetic and computational analysis revealed that even small amounts of collagen XVII have a remarkable effect on the phenotype. In contrast to complete null phenotypes, patients with only about 14% of collagen XVII of the control levels are still alive and independent and a large number of cell types and tissues exhibit a phenotype. Against this background, we addressed the pathogenesis of dermal abnormalities in KS by exploring cytotype profiles of KS keratinocytes and by characterizing KS skin fibroblasts in vitro, and by validating the findings in vivo in the skin of nine KS patients. We show that kindlin-1 deficient keratinocytes upregulate the expression of IL-24, IL-20, transforming growth factor-β (TGF-β), IL-1β, IL-12 and growth factor B (PDGFβ) and connective tissue growth factor (CTGF), and that KS fibroblasts exhibit an activated phenotype. These findings correlate with the presence of macrophages and of mediators of fibrosis, like α-smooth muscle actin, TGF-β, IL-6 and CTGF, as KS skin. Based on these data we predict that mutations in FERM1 gene cause epithelial cell stress and, as a stress response, activate cytokines that mediate local inflammation and fibrosis. The repeated cycles of epithelial cell stress, cytokine secretion, dermal inflammation and fibrosis underlie the phenotypic changes in tissue compartments in the skin. Once severe cytokine-mediated paracrine cell communication processes as novel phenotype modulators in KS and thereby yield a new starting point for development of therapeutic strategies.

P07.01
Skin compartments of different body areas harbour different amounts of mitochondrial deletions in aged wildtype mice

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Mutations of mitochondrial DNA play a causative role in aging of the skin including subcutaneous fat. We could previously show that the so called mitochondrial common deletion, a long-term marker for cellular stress, is increased in age-associated manner in subcutaneous fat from the facial area of mice deficient in the repair of oxidative stress. Here, we investigated whether these mutations are also present in aged wildtype mice and whether reduction of subcutaneous fat is also associated with increased mitochondrial deletions of DNA at other body sites than the face. To address this question, skin was collected from mice between 4 and 24 months of age, and also subcutaneous fat from the back (trunk), neck, chest, shoulder, lower and medial back, breast and pads of the fore limbs and hind limbs were collected. Subsequent metaphase analysis, denaturation, gel retardation and densitometric scanning of the mitochondrial DNA double-stranded ladder after BamHI digestion revealed a subcutaneous mitochondrial DNA double-stranded ladder, which is consistent with the increased number of mitochondrial deletions of DNA in subcutaneous fat. Subsequent analysis of mitochondrial DNA deletions in different skin compartments showed low levels of mitochondrial deletions and no significant age dependent increase of mitochondrial deletions in any investigated body areas. While epidermal and dermal compartments of all investigated body areas display only mild accumulation of mitochondrial DNA deletions and no age dependent increase of the mitochondrial deletion in subcutaneous fat. This is particularly increased in the pads of the fore limbs compared to all other body sites. These results indicate that mitochondrial DNA deletions are increased in the body compartments where prominent reduction of subcutaneous fat is observed in humans during the normal aging process.

P07.02
Whole genome (Exome) sequencing of Xeroderma pigmentosum patients reveals a finite and distinct pattern of mutations in pathophysiologically relevant genes

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Xeroderma pigmentosum (XP) is a rare autosomal recessive disease characterized by photosensitivity, sunburn at early age of exposure, growing sensitivity to UV light, and an increased number of cutaneous actinic keratoses. UV-induced DNA damage is not removed from the genome, the remaining photoproducts will give rise to UV-sensitivity mutations such as C→T and C→G to T→T transitions. While in XP patients it has been shown that isolated genes such as p53 harbor such mutations, thus far it was technically impossible to comprehensively investigate the whole genome. Therefore we performed a genome-wide and protein-coding oriented analysis that revealed even small amounts of collagen XVII have a remarkable effect on the phenotype. In contrast to complete null phenotypes, patients with only about 14% of collagen XVII of the control levels are still alive and independent and a large number of cell types and tissues exhibit a phenotype. Against this background, we addressed the pathogenesis of dermal abnormalities in KS by exploring cytotype profiles of KS keratinocytes and by characterizing KS skin fibroblasts in vitro, and by validating the findings in vivo in the skin of nine KS patients. We show that kindlin-1 deficient keratinocytes upregulate the expression of IL-24, IL-20, transforming growth factor-β (TGF-β), IL-1β, IL-12 and growth factor B (PDGFβ) and connective tissue growth factor (CTGF), and that KS fibroblasts exhibit an activated phenotype. These findings correlate with the presence of macrophages and of mediators of fibrosis, like α-smooth muscle actin, TGF-β, IL-6 and CTGF, as KS skin. Based on these data we predict that mutations in FERM1 gene cause epithelial cell stress and, as a stress response, activate cytokines that mediate local inflammation and fibrosis. The repeated cycles of epithelial cell stress, cytokine secretion, dermal inflammation and fibrosis underlie the phenotypic changes in tissue compartments in the skin. Once severe cytokine-mediated paracrine cell communication processes as novel phenotype modulators in KS and thereby yield a new starting point for development of therapeutic strategies.

P07.03
Vascular endothelial growth factor (VEGF) induces IL-23 expression in keratinocytes: a novel pro-inflammatory role for VEGF in psoriasis pathogenesis

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Angiogenesis has an important role in tumor growth and metastasis. However, vascular remodeling also occurs in many inflammatory and autoimmune diseases, including the chronic inflammatory skin disease psoriasis. The pro-angiogenic vascular endothelial growth factor (VEGF) might also act as a pro-inflammatory factor in the skin. A study was performed using psoriasis capture (NimbleGen EZ, Nimble Gen Systems, Waldkirchen, Germany) followed by next-generation sequencing. Expression profiling in PBLs enabled to screen the whole human genome, keeping the semantic changes in XP. Although analysis are still under way, preliminary data demonstrate an over-representation of cytosine and C→G to T→T transitions in skin cells (p<0.05 in T-tails). This study indicates that even in DNA repair deficient tissues with a promutator phenotype, only a limited number of somatic mutations is present in skin samples exposed to the relevant genotoxic stress. These findings could shed new light on the relations of DNA mutations and cancer susceptibility.

P07.04
TGF-beta integrin-dependent release of oxygen radicals from macrophages is required for TGF-beta1 activation in cutaneous wound repair

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Patients suffering from Leukozyt Adhesion Deficiency Syndrome type 1 (LAD1) with impaired β2 integrin expression and function due to mutations in the common β2 integrin chain (CD18) present with spontaneous skin alarations and severe wound healing disturbances. In a model of full thickness excisional wounds we previously found that disruption of the β2 integrin signaling pathway in CD18-/- mice leads to impaired wound healing primary keratinocytes (HKE) and fibroblasts. Similar to VEGF – which can be induced in keratinocytes and other cellular sources – upregulates pro-inflammatory IL-23 and IL-6 secretion in keratinocytes via p38 mitogen-activated protein kinase (MAPK) signaling. These results suggest that TGF-β1 which is released during inflammation and implicated in the repair of chronic wounds can be a novel pro-inflammatory cytokine. The role of TGF-beta1 and the underlying mechanisms of the pro-inflammatory function of VEGF is currently under investigation.

P07.05
AIM2 is overexpressed in psoriasis and an AIM2 inflammasome is active in human epidermal keratinocytes

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Although they are not professional immune cells, epidermal keratinocytes are at the first line of defense against invading pathogens and are able to initiate immune responses. In order to do so, they are equipped with danger sensors such as NLR-like receptors and inflammasome components. Inflammasome components are cytoplasmatic multi-protein complexes that upon activation lead to the processing of pro-inflammatory cytokines IL-1β and IL-18. Recently a novel inflammasome was characterized. AIM2 plays a central role in AIM2 inflammasome activation and its potential role in inflammatory skin diseases. We found increased AIM2 expression in psoriatic lesional skin compared to healthy or non-lesional skin. Additionally, active caspase1 and IL-1β production demonstrated inflammasome activity in lesional psoriatic plaques. In vitro, human epidermal keratinocytes secreted IL-1β and active caspase1 in response to IL-1β stimulation indicating the presence of AIM2 inflammasome. In addition, we investigated the expression of AIM2 in psoriatic lesion and in vitro IFN-x-gamma-induced AIM2 in primary epidermal keratinocytes. Finally, AIM2 deletion in keratinocytes from lesional psoriatic skin as a possible trigger of AIM2 activation in vivo. These data suggest that cytokine-driven AIM2 inflammasome induction is a possible mechanism for AIM2 activation in psoriasis lesions.
Treg deficient in IL-10 are insensitive to activation by ATP in vitro and fail to suppress contact hypersensitivity reactions. S. Ring, A. H. Enk and K. Mahnke. Department of Dermatology, University Hospital Heidelberg, 69115 Heidelberg, Germany.

The suppression of immune responses is guided by adenosine. R. Pruss, A. L. Beelen, A. K. Schroder and K. Schade. Department of Dermatology, University Hospital Heidelberg, 69115 Heidelberg, Germany.

Inhibitory effects of adenosine on immune cell activation have been described for over half a century. However, their mode of action on specific immune cell subsets is not fully understood. Here, we studied the activity of adenosine on murine T regulatory cells (Treg) and dendritic cells (DC) during the sensitization phase of CHS reactions. Intravenous injection of CD4+CD25+Foxp3+ regulatory T cells (Treg) into TNCB-sensitized mice before challenge suppressed the elicitation phase of murine contact hypersensitivity (CHS) reactions. In contrast, Treg deficient in IL-10 were insensitive to activation by graded doses of ATP in vivo and failed to suppress the elicitation phase of CHS reactions. Moreover, these data have to be taken into account when using IL-10-/- Treg in assays assessing the contribution of IL-10 to the T-regulated suppression in other disease models.


drastic cell death as a failure to produce TNF-α and IL-12 production when stimulated with LPS. In the presence of PG2 and similarly in the presence of cAMP-analogue, slanDCs displayed a strong capacity to undergo phenotypic maturation, however, their production of IL-12 and TNF-α was inhibited when stimulated after 12 and a second with a dose of LPS (0.001 mg/ml) and the stimulatory effect of LPS was reduced after 12 h with a second dose of LPS (0.001 mg/ml). The initial low level LPS challenge led to a dose-dependent reduction of TNF-α production and the inhibition of mRNAs encoding TNF-α and IL-12. These results strongly suggest that LPS-induced inhibition is not due to cytotoxicity or cytokine lysis on the single cell level. Interestingly, ET and 5z5 showed signs of an increase in maturity, which is taken together the phenotypic suppression of slanDCs particularly demonstrated in phenotypic, is profoundly and differently modulated by different microenvironmental factors as demonstrated for IL-10, PG2 and ET.


d to suppress the elicitation phase of CHS reactions. Moreover, these data have to be taken into account when using IL-10-/- Treg in assays assessing the contribution of IL-10 to the T-regulated suppression in other disease models.


dcategory of macrophage plasticity beyond the M1/2 dichotomy. We therefore assume that AD patients with autoreactive IgE may preferentially benefit from IgE-targeted therapies. To investigate the mode of action of IA in AD and following few reports on the presence of autoreactive forms of total IgE in AD patients, we determined autoreactivity of circulating and prolonged removal of skin-bound total IgE as well as reduction of dermal total IgE levels. Moreover, these data have to be taken into account when using IL-10-/- Treg in assays assessing the contribution of IL-10 to the T-regulated suppression in other disease models.


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PU.1, revealed that CD18-/- T-cells are shifted to Th17 differentiation already, while Th9 differentiation was demonstrated. Compared to wild-type controls, CD18-/- T-cells produced significantly more IL-17 upon stimulation. Differentiation by analyzing cytokine production. In addition, we determined expression of T-helper 2 (Th2) and T-helper 17 (Th17) cytokines. We observed an impaired upregulation of CCL21, CCR7 and IL-10 and an upregulation of IL-17 on IL-10 deficiency. The experiments revealed the existence of two subpopulations of IL-10 deficient mice with distinct states of differentiation, characterized as CCL21+/CCR7+/IL-10+ and CCL21+/CCR7-/IL-10-. In IL-10 deficient CCL21 mice, the mRNA expression of IFN-γ was not reduced in CCL21+ T cells as well as the levels of CD8+ T cells (CTL). CCL21 was increased compared to wildtype mice and additionally, these CTL expressed higher levels of activation and cytokine markers such as CCL5, granzyme B, IFN-γ, and the activating CCL21 receptor (NGR2B). Since during skin carcinogenesis the antigen presenting ability of T-cells migrates from the epidermal to the lymphoid compartment and induces the differentiation and activation of anti-tumoral effector cells from K14-RANKL tg mice, we observed an enhanced upregulation of MHC-I and CD83 and an increased expression of CD86, ILT-4 on DCs in K14-RANKL tg mice. Furthermore, we observed an impaired upregulation of RANK and RANKL in skin tumors of K14-RANKL tg mice compared to wildtype mice. The analysis of the up-regulated numbers of CTL in regional lymph nodes of K14-RANKL tg mice was used in further experiments as surface molecule for DC sorting. We are capable of separating CCL21+/IL-10+/CCR7+/CD83+ DC from CCL21+/IL-10-/CCR7-/CD83- DC. CD28 is the common beta-chain of CD80 and CD86 expressing cells. The expression of CD28 on lymphocytes is dependent on its presence during T-cell activation. As shown by immunohistochemistry, western blot analysis, ELISA, and transwell chemokinesis, the expression of CD28 is enhanced in SOD2+/− mice. In vitro, we observed an impaired expression of MHC-II and CD86. Immature SOD2+/− DCs produced increased proinflammatory IL-1 and IL-6 and chemokines CCL2 and CCL5 were upregulated. Fully matured DCs were efficiently upregulated MHC-II and CD86. Interestingly, in vivo contact hyper-responsiveness (CHS) was increased in SOD2+/− mice although SOD2+/+ mice showed no enhanced contact hypersensitivity (CHS) response. We observed that SOD2+/− T cells showed increased proliferation, even when stimulated with SOD2+/+ DC, possibly explaining the increased CHS in our model. In conclusion, SOD2+/− T cells are more likely to migrate to the draining lymph nodes than SOD2+/+ T cells. Thus, we suggest that SOD2+/− T cells might be a higher activation state of Tregs, as they are capable of inducing a more efficient immune response against tumor cells. Moreover, the increased expression of CD28 on SOD2+/− DCs might be responsible for the enhanced contact hypersensitivity (CHS) response. Therefore, SOD2+/− mice seem to be more resistant to the induction of CHS than SOD2+/+ mice. We conclude that SOD2+/− T cells might be a better candidate in the regulation of ‘inflamm-aging’ conveying both immunosuppressive and proinflammatory signals through alteration of DC and T cell functions.

**P078**

**Regulation of Th17 T-helper cell differentiation by beta-2 integrin CD18**

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**Abstract**

The development of skin cancer seems to be controlled by the immune system and innate as well as adaptive immune responses are crucial for the induction of skin malignancy. Evidence from our laboratory and others has shown that cutaneous squamous cell carcinoma (K14-RANKL tg) mice have shown that cutaneous over-expression of RANK ligand (RANKL) (P088) resulted in the development of skin tumors. These skin tumors were strongly reduced in K14-RANKL tg mice expressing human CD18 in the skin, indicating that skin tumour development and tumor growth in tg mice could be attributed to increased RANK-RANKL signaling due to barrier disruption. This may also be part of a compensatory protection mechanism avoiding excessive senescence upon barrier disturbance may be part of a compensatory protection mechanism avoiding excessive senescence. The induction of tolerance via generation of Treg appears to be a mechanism to control skin cancer progression. In order to understand the mechanism of the development of skin cancer we have focused on the role of skin-derived DC. We have characterized these DC in terms of their phenotype and functional ability to suppress T-cell immunity. In this study we have analyzed the expression of CD18 in the epidermis and observed that the expression of CD18 was significantly increased in K14-RANKL tg mice compared to wildtype controls. We have also shown that CD18 expression was increased in K14-RANKL tg mice in the skin tumor development and tumor growth in tg mice could be attributed to increased RANK-RANKL signaling due to barrier disruption. This may also be part of a compensatory protection mechanism avoiding excessive senescence. The induction of tolerance via generation of Treg appears to be a mechanism to control skin cancer progression. In order to understand the mechanism of the development of skin cancer we have focused on the role of skin-derived DC. We have characterized these DC in terms of their phenotype and functional ability to suppress T-cell immunity. In this study we have analyzed the expression of CD18 in the epidermis and observed that the expression of CD18 was significantly increased in K14-RANKL tg mice compared to wildtype controls. We have also shown that CD18 expression was increased in K14-RANKL tg mice in the skin tumor development and tumor growth in tg mice could be attributed to increased RANK-RANKL signaling due to barrier disruption. This may also be part of a compensatory protection mechanism avoiding excessive senescence. The induction of tolerance via generation of Treg appears to be a mechanism to control skin cancer progression. In order to understand the mechanism of the development of skin cancer we have focused on the role of skin-derived DC. We have characterized these DC in terms of their phenotype and functional ability to suppress T-cell immunity. In this study we have analyzed the expression of CD18 in the epidermis and observed that the expression of CD18 was significantly increased in K14-RANKL tg mice compared to wildtype controls. We have also shown that CD18 expression was increased in K14-RANKL tg mice in the skin tumor development and tumor growth in tg mice could be attributed to increased RANK-RANKL signaling due to barrier disruption. This may also be part of a compensatory protection mechanism avoiding excessive senescence.
P001 Potential psoriatic autoantigen may result from cross-reactive streptococcal-specific immune-responses

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Potential psoriatic autoantigens may result from cross-reactive streptococcal-specific immune-responses. Our group has previously shown the existence of a novel autoantigen, termed psoriasin-1 (P1), which is expressed in psoriatic lesional skin and reactive T cells. However, its immune-specificity is still unclear. In the present study we performed mass spectrometry-based analysis and immunohistochemistry to investigate the existence and the immune-specificity of P1 in psoriatic lesional skin and reactive T cells.

P002 Single cell analysis confirms antigen-specific clonal T cell expansions within psoriatic skin lesions

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Today, psoriasis is considered as a T-cell mediated autoimmune disease. A clonal antigen-specific T-cell response against a psoriatic-specific immune-responses has been proposed by T-cell receptor (TCR) analysis of the infiltrating inflammatory in psoriatic skin. To define the clonal nature of the lesional psoriasis T-cell response we developed a RT-PCR based method to characterize the paired TCRα and TCRβ chain rearrangements from single T-cells, which had been isolated from lesional psoriatic biopsies using magnetic beads coated with CD4 or CD8 monoclonal antibodies.

Single cell TCR analysis identified multiple CD4+ or CD8+ T-cells with identical TCR rearrangements in both the lesional psoriasis T-cell population. This suggests that a clonal T-cell expansion was found in single T-cells, which had been isolated from lesional psoriatic skin and reactive T cells.

P003 Enhanced primary but not memory anti-viral immune responses by interferon γ

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It is widely accepted that acquired T cell responses are critical to host defense against microbial pathogens. However, the role of interferon γ (IFN-γ) in human T cell-mediated immunity to microbial infection is not clearly defined.

In the present study, we have investigated potential differences in the induction and maintenance of primary and memory type 1 T cell responses to viral infection, specifically in IFN-γ deficient mice. To this end, we infected IFN-γ deficient and wildtype mice with murine cytomegalovirus (MCMV) and measured the induction of primary and memory type 1 T cell responses in both groups of mice.

We observed that IFN-γ deficient mice show normal induction of primary type 1 T cell responses. However, IFN-γ deficient mice were unable to maintain these responses. These results suggest that IFN-γ is important for the maintenance of memory type 1 T cell responses, but not for their induction.

P004 Thy-1 (CD90) regulates extravasation of inflammatory cells during skin inflammation

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Thy-1 (CD90) is required for human T cell mediated immunity to microbial infection. It is widely accepted that acquired T cell responses are critical to host defense against microbial pathogens. However, the role of interferon γ (IFN-γ) in human T cell-mediated immunity to microbial infection is not clearly defined.

In the present study, we have investigated potential differences in the induction and maintenance of primary and memory type 1 T cell responses to viral infection, specifically in IFN-γ deficient mice. To this end, we infected IFN-γ deficient and wildtype mice with murine cytomegalovirus (MCMV) and measured the induction of primary and memory type 1 T cell responses in both groups of mice.

We observed that IFN-γ deficient mice show normal induction of primary type 1 T cell responses. However, IFN-γ deficient mice were unable to maintain these responses. These results suggest that IFN-γ is important for the maintenance of memory type 1 T cell responses, but not for their induction.

P005 Linking BOS production and HA degradation – a crucial role for the generation of endogenous ligands in CHS responses to contact sensitizers

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Contact hypersensitivity (CHS) is typically elicited by contact with chemical allergens that trigger an innate immune response and are capable of inducing antigen-specific T cell responses in the draining lymph nodes. In the present study, we investigated the role of the innate immune system in the generation of endogenous ligands that trigger antigen-specific T cell responses in the draining lymph nodes during CHS.

We found that the innate immune response was essential for the generation of endogenous ligands that trigger antigen-specific T cell responses in the draining lymph nodes during CHS. These results suggest that the innate immune system plays a crucial role in the generation of endogenous ligands that trigger antigen-specific T cell responses in the draining lymph nodes during CHS.
Identification of triptolide as a potential aryl hydrocarbon receptor antagonist in memory T cells

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To test whether exposure to triptolide affects differentiation of naïve human T cells to effector cells, we added triptolide and IFN-γ during the in vitro differentiation of naïve T cells. Comparison of Th1 differentiation in naïve T cells by intracellular staining and ELISA after the addition of IFN-γ with different concentrations of triptolide showed desired differences in IL-17, IL-23 and IL-22 production in a dose-dependent manner. Realtime-PCR demonstrated a strong down-regulation of IFN-γ, IL-17A and IL-22 in human T cells, as well as an induction of genes encoding xenobiotic metabolizing cytochrome P450 enzymes such as CYP1A1 and CYP1B1.

Stable suppression of protein expression via retroviral transduction with shRNA as method to alter T cell function using the example of PD-1 in tumour-specific T cells

V. H. Voelkel1,2, J. J. Whitehouse1,2, S. C. Montandon1,2, P. L. Rice1,2, N. K. MacKenzie1,2, J. H. Martin1,2, T. Schumacher1,2 and C. Blank1,2

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the innate immune response in CHS. We analysed whether extracellular ATP, released from stressed cells, activates the inflammatory pathway and its effect on the formation of CLE ATP receptor such as P2X7 receptor. This receptor activates the NLRP-inflammusome that processes pro-IL-1β and pro-IL-18 via caspase.

We assessed the role of the ATP receptor P2X7 in CHS, using CRISPRi or P2X7-/- mice. In addition, we investigated the involvement of the NLRP7 inflammasome in the ATP response using ASC-/- and NLRP7-/- mice in our setting. In addition, ATP production in situ and IL-1β processing in dendritic cells (DC) as well as in T cells en route were analysed. Here, we demonstrate that extracellular ATP is a trigger for CHS. Moreover, P2X7-/- mice have the ability to sense both wild type and P2X7-deficient receptors against the contact sensitizer TCSN. Suppression of P2X7 signalling by the antagonists SB203580 or SB202190 or neutral or extracellular ATP by the ATP degrading enzyme apyrase can prevent CHS. In vivo immunohistochemistry imaging revealed that treatment of mice with contact-sensitizers induces ATP release from skin cells. LPS primed P2X7-deficient BMCs did not release mature IL-1β in response to ATP treatment. Pretreatment with the P2X7-independent inflammasome activator anamol restored the sensitizing potential in P2X7-/- mice, suggesting a role for IL-1β in vitro. Blood samples from P2X7-/- mice carrying over the P2X7 antagonist Amapalone also predicted CHS. These findings clearly show that contact allergens such as TCSN and enzobine indirectly activate PRR signaling via agonistic uricosuric. Up to now, ACD treatment only comprises symptomatic therapy with anti-inflammatory drugs such as clobetasol. Inhibition of P2X7 signaling is an important step towards the new causative treatment by specific innate immune modulation.

P105 (V16)

Spontaneous inflammatory blisters in a new passive transfer model of bullous pemphigoid in adult mice

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1Department of Dermatology, University of Freiburg, 79104 Freiburg, Germany; 2Department of Dermatology, University of Immunofluorescence microscopy, deposits of rabbit IgG and murine complement C3 at the DEJ were detected. Specific antibodies, mice were examined over a period of 20 days. The lesions, including blisters, erythema, and erosions with crusts developed at distant predilection sites such as ears, snouts and limbs. BALB/c and C57BL/6 mice were injected on the right flank with the anti-GBM antibodies. Antibody titers were determined by ELISA. Histopathological analysis of lesional skin revealed dermal-epidermal blister formation, and associated with very low complement deposition and granulocyte recruitment. It is known that anti-GBM antibodies initiate local complement activation and the recruitment of inflammatory cells, which are prerequisites for the formation of bullous pemphigoid.

Conclusions: The present findings suggest that the combination of immunoadsorption and rituximab induces both a rapid clinical remission and leads to a long-term control of disease activity in severe refractory pemphigus patients.

P107

Impact of keratinocyte-derived type III interferon (IFN-J) in cutaneous lupus erythematosus and related autoimmune disorders

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Background: Type I interferons (IFN) have been shown to play a central role in the pathophysiology of lupus erythematosus (LE). The recently discovered type III IFNs (IFN-Js) share several functional similarities with type I IFNs. Since IFN-J lambda has been shown to act primarily on epithelial cells, we investigated the function of type III IFNs in the pathostatic network in cutaneous LE (CLE) and related disorders.

Methods: We characterized the ability of keratinocytes to produce IFN-J lambda in response to immunostimulatory nuclear acids was determined in cell culture experiments using epidermal explants and primary human keratinocytes. IFN-J lambda expression in skin biopsies and IFN-J lambda serum levels were measured in patients with cutaneous LE, dermatomyositis (DM), lichen planus (LP) and healthy controls by immunohistochemistry and ELISA. The functional impact of IFN-J lambda on skin pathology recruitment was assessed in cell migration assays.

Results: IFN-J lambda and the IFN-J lambda-antagonist are strongly expressed in active skin lesions of CLE, LP and DM. IFN-J lambda-expressing keratinocytes are primarily seen in LP, LE and CLE lesions. Second, enhanced IFN-J lambda levels could be measured in the serum of patients with active disease: Third, the lesion expression pattern of IFN-J lambda correlates with that of the type I IFN lambda. Fourth, the results provide evidence related to the recruitment of inflammatory cells and are associated with the formation of cutaneous LE lesions. Our results provide insights into the role played by type III IFNs in CLE skin disease. Since the levels of systemic type III IFNs vary between patients, we propose that type III IFN is a major mediator of disease in CLE skin lesions. Second, enhanced IFN-J lambda levels could be measured in the serum of patients with active disease: Third, the lesion expression pattern of IFN-J lambda correlates with that of the type I IFN lambda. Fourth, the results provide evidence related to the recruitment of inflammatory cells and are associated with the formation of cutaneous LE lesions. Our results provide insights into the role played by type III IFN in CLE skin disease. Since the levels of systemic type III IFNs vary between patients, we propose that type III IFN is a major mediator of disease in CLE skin lesions.

Conclusion: Our observations provide several lines of evidence that keratinocyte-derived IFN-J lambda plays a role in the pathophysiology of CLE. The results underline the need to develop new therapeutic strategies for CLE skin lesions. Second, enhanced IFN-J lambda levels could be measured in the serum of patients with active disease: Third, the lesion expression pattern of IFN-J lambda correlates with that of the type I IFN lambda. Fourth, the results provide evidence related to the recruitment of inflammatory cells and are associated with the formation of cutaneous LE lesions. Our results provide insights into the role played by type III IFNs in CLE skin disease. Since the levels of systemic type III IFNs vary between patients, we propose that type III IFN is a major mediator of disease in CLE skin lesions.

P108

Poly IC induces IFN lambda expression in keratinocytes via endosomal (TLR-dependent) and cytosolic (TLR-independent) pattern recognition receptors

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Background: The synthetic immunostimulatory molecular poly IC is a strong inducer of type III interferon production (IFN-J lambda) in keratinocytes. Interestingly, this stimulation, as well as infection of keratinocytes with vesicular stomatitis virus, has an only minor effect on the levels of other interferons (alpha, beta, gamma), suggesting that IFN-J lambda is the major inducer of these cells. Poly IC has been found to have the capacity to stimulate innate immune responses via the TLR3 pathway.

Methods: Human and murine keratinocyte cell lines, including MDAs-knockout cells, were cultured and stimulated with different synthetic lipids of endosomal and cytosolic IFN-Js. Additionally, poly IC and poly XDRP, a poly IC-activating agent, were used to induce type III cytosolic and endosomal IFN-J pathway. RT-PCR and ELISA were used as readouts.

Results: Chloroquine reduces the poly IC-induced IFN lambda expression by half; the same effect was seen using inhibiting siRNA for IFN-J lambda. Poly IC-induced IFN lambda expression was also reduced by anti-IFN-J lambda antibody. Similar results were found for the prototypical cytopathic infections including influenza and rheumatoid arthritis.

Conclusion: Poly IC induces the expression of type III interferons via TLR-dependent and cytosolic pattern recognition pathways. Our results demonstrate that different innate immune response pathways drive the induction of keratinocyte IFN lambda expression and support the view that the type III IFN system has an outstanding role for the innate immune response of these cells.

P109

The Molecular Profile of Psoriatic Skin in Responders to Ustekinumab or Etanercept Following Twelve Weeks of Treatment: Results from the ACCEPT Trial

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Question: To assess the impact of p10 cytokines (IL-12p70,23) or TNF alpha blockers on resident and inflammatory cells and on the expression of gene pathways that may drive chronic immune activation and inflammation in the skin.

Methods: In ACCEPT, a randomized, controlled clinical trial, the efficacy of ustekinumab and etanercept in 904 patients with moderate-to-severe plaque psoriasis through wk 12. Skin samples were processed in a subset of patients at baseline, wk 12 and 12. Microarray analysis (Affymetrix U133a2 array) comparing non-lesional skin (n = 85) to lesional skin (n = 85) at baseline and wk 12 using several thousand probe sets differentially expressed (fold change, ERH, P < 0.05) in lesional skin.

Results: Patients responding to each agent (PASI, n = 21 for stentor, n = 19 ustekinumab) had significant changes in approximately 4000 transcripts compared to untreatered lesions, indicating significant reprogramming of pathways related to immune function: keratin 6a and 16, and immune defense products (cytokine-modulated genes in keratinocytes), were commonly reported by ustekinumab recipients. The genes down-regulated at wk 12 by ustekinumab overlap with nine of the top 10 genes down-regulated by etanercept at wk 12; only two of the top 10 genes up-regulated overlap with nine of the top 10 genes up-regulated by etanercept (THBG, THRSX): this comparison. The genes up-regulated by ustekinumab include a subset of keratin structural proteins indicating a unique effect of ustekinumab on keratinocytes.

Conclusions: Aggregation of common and unique effects of ustekinumab and etanercept defines critical pathways involved in psoriasis pathogenesis and a successful therapeutic response. Broad genomic
assessments provide an independent way to judge to the extent to which disease pathology can be reversed by effective therapeutics.

\[ \text{P110} \]

TNF-α mediates a delayed anti-inflammatory feedback mechanism in the response of monocytes to LPS


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Lipopolysaccharide (LPS) is the major component of the outer membrane of Gram-negative bacteria. Recognition of LPS by monocytes initiates a rapid release of inflammatory mediators resulting in activation of leukocytes. However, a reliable analysis of the delayed response of monocytes elicited by LPS is not yet done.

Under these experimental conditions, lipopolysaccharide transcripts are mainly regulated in a MAP-kinase p38-dependent manner. Interestingly, statistical analysis of microarray data indicated a significant up-regulation of cytokine genes and TNF, accompanied by a cell cycle arrest in STAT1-deficient cancer cells, and TNF failed to arrest cell cycle in STAT1-/- (TNF pathway) and RIP1-Tag2 cells. Importantly, fumarates protect mice from experimental autoimmune encephalomyelitis (EAE). Anti-inflammatory type II DC that promote IL-4-producing Th2 cells and suppress Th1 and Th17 cell responses may be involved in the suppression of EAE.

\[ \text{P111} \]

Cytoinkyte-induced cell cycle arrest in isolated cancer cells

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Even though most established tumor immunotherapies are based on tumor cell destruction by cytotoxic T lymphocytes (CTLs) or on induction of an anti-inflammatory monocyte phenotype which actively suppresses pro-inflammatory responses from other immune cells.

Intracellular Caspase-8 expression by these antigen-specific T cells was significantly reduced when compared to wild-type T cells. T helper 1 (Th1) cells. RIP1-Tag2 mice undergo multistage carcinogenesis by expressing the oncoprotein T antigen 2 (Tag2) of the Simian Virus 40 under control of the rat insulin promoter (RIP1). Tag2 mice were stained for CD137 receptor and transferred to macrophages we observed a significant reduction of their capacity to inhibit the release of pro-inflammatory cytokines by monocytes. Therefore, our data indicate an important role of TNF-alpha in the generation of an anti-inflammatory monocyte phenotype during the late phase of monocyte activation by LPS. This may explain the use of TNF-alpha as a potential treatment of sepsis. Improving our understanding of this anti-inflammatory feedback mechanism in the response to pathogenic stimuli is important for the development of new therapeutic regimens for infections as well as for immune disorders.

\[ \text{P112 (V34)} \]

Both loss of tolerance to type VII collagen and autobiotically-induced tissue injury are genetically controlled in experimental EBA


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Epidermolysis bullosa acquisita (EBA), an autoimmune blistering disease, characterized by antibodies to type VII collagen (COL7). EBA can be induced in mice either by transfer of anti-mouse COL7 IgG or by immunization with a fragment of mouse COL7. In contrast to other autoimmune diseases, e.g. rheumatoid arthritis, little is known about the genetic susceptibility for EBA. We therefore used two EBA susceptible and two resistant strains to address the role of both disease induction and autobiotically-induced tissue injury (1g2) transfer are genetically controlled in these experimental models. Despite 10 and 20 weeks of disease induction, the susceptibility to EBA development varied among the inbred mouse lines. Specifically, C57BL/6 mice were highly susceptible to EBA induction by antibody transfer, while the other strains, including BALB/c or DBA/2 mice, were completely protected. We then used publically available genotyping data from those inbred mouse lines to identify gene loci associated with autobiotically-induced tissue injury. Indeed, this analysis identified several loci controlling autobiotically-induced tissue damage during EBA development: EBA only occurs in those strains, where both, autobiotically-induced tissue injury and autobiotically-induced tissue injury are genetically controlled in experimental EBA. The identified gene pools provide further insight into the pathogenesis of this disease which may ultimately facilitate the development of novel therapeutic strategies.

\[ \text{P113} \]

Mast cells in psoriatic lesions express CD3 receptor


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Our data illustrate that maternal exposure with BPA promotes the development of experimental asthma in mouse pups and that BPA affects the clonal expansion of allergen-specific T cells in the lungs. The role of STAT3 in this context has not yet been sufficiently assessed. Therefore, we assessed the role of STAT3 in the development of experimental asthma triggered by allergens.

\[ \text{P114} \]

Effect of Bisphenol A on dendritic cell maturation and T cell plasticity

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Bisphenol A (BPA) is a main estrogen-seomoon used in the plastic industry. It has been shown that maternal exposure with BPA promotes the development of experimental asthma in mouse pups and that BPA affects the clonal expansion of allergen-specific T cells in the lungs. The role of STAT3 in this context has not yet been sufficiently assessed. Therefore, we assessed the role of STAT3 in the development of experimental asthma triggered by allergens.

We found a significant expression of the proliferation of the isolated anti-TNF-α tagged T cells in vitro or in vivo, and transfected by a cell cycle in G1. On the other hand, none of the two cytotoxic cancer cell lines increased of mc28 cells and can significantly be blocked by anti-CD137 antibody. The effects of anti-CD137 in the specific and strictly required both STAT1 and TNFR1. TNF failed to block cell growth, and STAT1-deficient cancer cells, and TNF failed to block cell growth, and STAT1-deficient cancer cells. Using PCR arrays we found that TNF-α strongly affects the expression of cell cycle regulatory genes, mainly regulating G1/S progression.

Together, our data suggest that Tag1Th1-mediated immunity controls TNF-α- and TNF-α cell cycle arrest in the absence of cancer cell death.

\[ \text{P115} \]

Psoriatic cytokines induce insulin resistance in T-lymphocytes


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Psoriasis is a chronic inflammatory disease of the skin and joints caused by a complex interplay of genetic and environmental factors. The pathogenesis of this disease is multifaceted with involvement of the immune system.

In summary, our findings show that both, loss of tolerance and autoantibody-induced tissue injury of CD137 receptor. Therefore mast cells activated via CD137 receptor may be an important source for secretion of proinflammatory cytokines such as IL-6 and TNF-α, especially in the early phase of development of psoriatic plaques.

\[ \text{P116} \]

Fumarate-induced HO-1 differentially regulates the expression of IL-23 and IL-12

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Fumarate treatment induced HO-1 and this was associated with a decrease in IL-23 mRNA in DCs and IL-12 mRNA in T cells. In previous studies, BPA-induced expression of IL-12p35 was found to be independent of STAT3. In this study, the original source of BPA was not yet characterized, and in murine DCs and mouse cells, BPA-induced expression of IL-23 was analyzed by flow cytometry. Low dose treatment with BPA did not influence the expression of interferon and IL-12p40, but high dose BPA on dendritic cell maturation and T cell plasticity.

The expression of HO-1 on the immunization-induced T cell response in naive CD4+ T cells (MoDC) and naïve CD4+ T cells was slightly reduced.

These results demonstrate that BPA does not influence MoDC maturation, however, it may influence T cell homing properties.
P117

Connecting expression of the immunological cell stress indicator ULBP2 to the tumor suppressor activity of p53

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The activating immunoregulator NKG2D, expressed on Natural Killer (NK) cells and different subsets of T cells, and in its ligands MHC class I chain-related (MIC) and ULBP binding protein (ULBP) molecules, play a key role in tumor immunity. A variety of malignancies show reduced expression of MIC and ULBP molecules, sensitizing them for NK cell and T cell-mediated cytolysis. However, tumors can subvert NKG2D-mediated immune-surveillance by ligand shedding. In sera from melanoma patients we detected increased levels of both NKG2D ligands, Interestingly, elevated soluble ULBP2 in sera from patients with multiple myeloma correlated positively with progression between both ligands exist with respect to expression and clinical significance. On the basis of these findings we set out to define signals that control ULBP2 expression in melanoma. We observed that treatment of the tumor cells with different chemotherapeutics, like cephalosporin and doxorubicin, strongly upregulate the surface expression of both ULBP2 and myeloma cells. ULBP2 induction was detectable also at the levels of total cellular protein and specific mRNA. Blockade of ATM kinase activity by the specific inhibitor KU-55933 abrogated ULBP2 induction, pointing to an involvement of DNA damage signaling pathways in ULBP2 regulation. To test whether ULBP2 induction is p53 dependent, we analyzed the ULBP2 mRNA and protein levels in HCT 116 wt and HCT116p53-/- cells upon cephalotaxin treatment, respectively. We observed that upon cephalotaxin treatment, levels on HCT 116 p53-/-, albeit to a lesser extent when compared to HCT 116 wt. In summary, our data demonstrate a strong clinical significance of ULBP2 expression in melanoma and point to an involvement of p53 in ULBP2 regulation.

P118

Curcumin protects from autoimmune disease by modulating DC differentiation and suppressing Th1 and Th17 cells

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Curcumin is a polyphenolic natural product isolated from the rhizomes of Cinnamomum zeylanicum, a spice commonly used in Indian and Asian cuisine. Curcumin is a multi-targeting compound with a broad range of biological effects ranging from anti-inflammatory activity to inhibition of tumor growth. The molecular mechanisms underlying the therapeutic properties of curcumin are not completely understood. To investigate the effects of curcumin on dendritic cell (DC) differentiation and function we studied the effects of curcumin on DC subsets in vitro and in vivo in experimental autoimmune encephalomyelitis (EAE). The results suggest that curcumin reduces the pro-inflammation Th1 and Th17 cell response. The main findings are: First, the elucidation of the effects of curcumin on Th1 and Th17 cells using mixed leukocyte reaction (MLR) assay. Second, the analysis of the effects of curcumin on DC differentiation and function in vitro and in vivo. Third, the analysis of the effects of curcumin on DC subsets in experimental autoimmune encephalomyelitis (EAE). The results show that curcumin reduces the pro-inflammatory Th1/Th17 response and inhibits the development of EAE. In conclusion, curcumin is a promising therapeutic agent for the treatment of autoimmune diseases.

P119

The CD10/CD18 lymphocyte adhesion molecule – insight into the pathogenesis of a complex disease

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Pathogens are a frequent threat to the human body. Infections can be caused by bacteria, viruses, fungi, and parasites. The human immune system is the first line of defense against these pathogens. The CD10/CD18 lymphocyte adhesion molecule plays a crucial role in the immune system by mediating cell adhesion and migration. In this study, we investigated the CD10/CD18 lymphocyte adhesion molecule in the pathogenesis of rheumatoid arthritis (RA). Our findings suggest that the CD10/CD18 lymphocyte adhesion molecule may be involved in the pathogenesis of RA. The results of this study provide new insights into the pathogenesis of RA and may have implications for future research and treatment.

P120

Staphylococcus aureus adherence to human endothelial cells depends on von Willebrand factor and shear flow

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1Institute of Hygiene and Microbiology, University of Dermatology, Venerology and Allergology, Experimental Dermatology, Medical Faculty Mannheim, Molecular Medicine, Goethe-University, Frankfurt am Main, Germany; 2Department of Dermatology, which in conjunction with the CD18 hypomorphic mutation are responsible for the dysregulation of these adhesion molecules, play a key role in the pathogenesis of a complex disease. Our findings suggest that von Willebrand factor and shear flow may be involved in the adherence of Staphylococcus aureus to human endothelial cells. The results of this study provide new insights into the pathogenesis of Staphylococcus aureus infection and may have implications for future research and treatment.

By contrast, the skin of 80-100% of patients with atopic dermatitis is colonized with S. aureus and S. aureus infection is the most common cause of acute and chronic skin infections such as endocarditis or sepsis. In the setting of skin infections caused by Staphylococcus aureus, the skin is the site of infection and the skin barrier is affected. The skin is a complex organ composed of keratinocytes, fibroblasts, and immune cells. The skin barrier is crucial for maintaining homeostasis and protecting the body from pathogens. In the setting of skin infections caused by Staphylococcus aureus, the skin is the site of infection and the skin barrier is affected. The skin is a complex organ composed of keratinocytes, fibroblasts, and immune cells. The skin barrier is crucial for maintaining homeostasis and protecting the body from pathogens. In the setting of skin infections caused by Staphylococcus aureus, the skin is the site of infection and the skin barrier is affected. The skin is a complex organ composed of keratinocytes, fibroblasts, and immune cells. The skin barrier is crucial for maintaining homeostasis and protecting the body from pathogens. In the setting of skin infections caused by Staphylococcus aureus, the skin is the site of infection and the skin barrier is affected. The skin is a complex organ composed of keratinocytes, fibroblasts, and immune cells. The skin barrier is crucial for maintaining homeostasis and protecting the body from pathogens. In the setting of skin infections caused by Staphylococcus aureus, the skin is the site of infection and the skin barrier is affected. The skin is a complex organ composed of keratinocytes, fibroblasts, and immune cells. The skin barrier is crucial for maintaining homeostasis and protecting the body from pathogens.
Induction of an adverse immune response towards an implant still represents the major threat of suc-
cessful biomaterials. For this reason, strategies that enhance the control of the innate immune re-
sponse have been developed to equip biomaterials with immunomodulating capabilities. One strategy is the use of extracellular matrix (ECM) components to modulate coating. Here, in this study we address the immunomodulatory effects of artificial ECM (aECM) that were generated utilizing the natural self-assembly potential of collagen in combination with either hydro-
phobic (HA) or chemically crosslinked (CC) ECM. Both glycosaminoglycans (GAGs) were additionally modified by attaching of sulphate groups at low (5%) and high levels (30%) providing binding sites for endogenous growth factors and inflammatory mediators. Dendritic cells (DC) are key players of innate and adaptive immunity. They exert immuno-regulatory functions by controlling the intensity and duration of healing by creating a naturally surrounding for the host cells.

In the present study we address the immunomodulatory effects of artificial ECM (aECM) that were generated utilizing the natural self-assembly potential of collagen in combination with either hydro-

dermally crosslinked (HA) or chemically crosslinked (CC) ECM. Both glycosaminoglycans (GAGs) were additionally modified by attaching of sulphate groups at low (5%) and high levels (30%) providing binding sites for endogenous growth factors and inflammatory mediators. Dendritic cells (DC) are key players of innate and adaptive immunity. They exert immuno-regulatory functions by controlling the intensity and duration of healing by creating a naturally surrounding for the host cells.

In this model immature immune signals were sufficient to elicit full blastogenesis in response to low doses of antigen. Surprisingly, co-factor dependent adjuvants by aECM was independent of matured DCs and fully dependent on DCs. Our results for the first time show a mechanism of how infections trigger aB cells. This allows for a major clinical importance for the management and preven-
tive measures in patients with co-factor induced adjuvant and may lead to new therapeutic strate-
gies.

Immunomodulatory effects on dendritic cells of biomaterial coatings based on artificial extracellular matrices (aECM)

P212

Thymic stromal lymphopoeitin enhances the Th2 inducing capacity of human plasmacytoid dendritic cells.

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Increased numbers of the recently defined IL-23 producing Th2 cells have been identified in inflam-

matory skin lesions, including atopic dermatitis. Thymic stromal lymphopoeitin (TSLP) is classically present in atopic inflammation; however, the impact of a TSLP-dominated inflammatory milieu on Th2-differentiation has not been analyzed in detail since human plasmacytoid dendritic cells (pDC) proved to be superior to other DC subsets in prim-

ing Th2, the proteins of activated human pDC and their priming capacity on naive T cells were ana-

lyzed as a function of TSLP.

Immature pDC and control DC were isolated from peripheral blood of healthy donors. Toll-like recep-
tors (TLR) were activated with respective TLR ligands. GPICOS analysis of pDC by TLR9 resulted in IL-

6 production. Th2 polarizing capacity of TLR9 was boosted by TLR7/8 agonist. TLR2 agonist further enhanced T2-polarizing activity of pDC and TLR9. We therefore analyzed pDC from healthy donors and found that T2-polarizing activity of pDC in response to TLR7/8 agonists is significantly higher than in response to TLR9.

Generation of a DEC205+ specific single chain fragment variable (scFv)

Toxin to deplete tolerance inducing dendritic cells

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Generation of a DEC205+ specific single chain fragment variable (sFcV)

toxin to deplete tolerance inducing dendritic cells

DEC205+ is expressed on Langerhans cells and plasmacytoid dendritic cells (pDC). In addition, pDC are known to produce type I interferons (INF-α/β) and induce T cell differentiation towards IFN-γ polarizing capacity. A recently developed experimental vaccine against melanoma is based on the use of an IFN-α inducer. This approach has been successful in preclinical vaccination studies but failed in clinical trials. Therefore, we investigated the potential of a IFN-α antagonist for the treatment of melanoma.

We investigated SDC 1 and SDC 4-knockout mice compared to wildtype mice in the murine contact hypersensitivity (CHS) model. SDC deficiency does not influence cutaneous injury. However, the CHS response was abolished or partially suppressed in SDC-deficient mice.

The CD4+ T cells phenotype was assessed by analyzing expression of DC maturation surface markers, cytokine profile and allostimulatory activity in a mixed lymphocyte reaction. We find that collagen alone provokes DC activation. Culture of DC on collagen induces up-regulation of HLA class I and II and increased expression of IL-12 and IFN-γ. In all conditions we found T2-polarizing activity of DC and further experiments will reveal the role of this DC subpopulation in tolerance and immunity.

Acute pulmonary anaphylaxis is induced by intravenous injection of ovalbumin (OVA) sensitized mast cells (MC) into anaesthetised mice. This model allows investigation of MC co-factor dependent anaphylaxis. In this model innate immune signals were sufficient to elicit full blastogenesis in response to low doses of antigen. Surprisingly, co-factor dependent adjuvants by aECM was independent of matured DCs and fully dependent on DCs. Our results for the first time show a mechanism of how infections trigger aB cells. This allows for a major clinical importance for the management and preven-
tive measures in patients with co-factor induced adjuvant and may lead to new therapeutic strate-
gies.
Conclusion: We established a model to study the effects of specific OPN binding motifs on DC functions and could thereby be an interesting new strategy for cancer therapy, especially in combination with current immunotherapy protocols.

P130

Soluble CD83 promotes tolerance induction to skin and heart transplants in the mouse

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Background: Within the skin, cell structure, composition and stiffness of their environment by integrin-extracellular matrix (ECM) interactions, initiating bi-directional signaling that controls adhesion, proliferation, differentiation and apoptosis. Osteopontin (OPN) contains a central RGD-peptide to interact with alphav-beta3-integrins, known to mediate adhesion, migration and survival of various cell types. However, the exact mechanism by which CD83-positive monocytes induce acute rejection and delayed heart graft survival for more than 100 days. Taken together, these data indicate that CD83 can provide a promising therapeutic approach to induce tolerance in clinical transplantation.

P131

Influence of specific OPN binding motifs on dendritic cell functions by nanostructured, biofunctionalized surfaces

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Methods and Results: RGD-dependent integrin lateral clustering was studied using nanopatterned polyethylene glycol (PEG) hydrogel surfaces, containing RGD-biofunctionalized gold nanoparticles on an acute rejection and achieved graft tolerance with indefinite survival for more than 100 days. Taken together, these data indicate that CD83 can provide a promising therapeutic approach to induce tolerance in clinical transplantation.

P132

Providing T-cell help to dendritic cells to induce better anti-tumor immune responses

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Abstracts
Abstracts

CD4 signaling domain sometimes improved the CAR surface expression, and always improved the cytokine secretion and surface expression of the CAR-TCR. Furthermore, we were able to prove that both TCRs were efficiently transfected into the T cells and the CD8+ T cells, which were transfected with two different TCRs can represent a new tool to study TCR functionality and might be used in the adoptive immunotherapy of cancer patients.

P139 Generation of dual-specific CD8+ T cells against HIV-1 by transfection of TCR-encoding RNA

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HIV-1 specific CD8+ cytotoxic T lymphocytes (CTL) play an important role in the immune response against HIV. Unfortunately, many patients are unable to generate a strong immune response against this virus. For these patients, adoptive transfer of T cells reprogrammed with an HIV-specific TCR is a potential immunotherapeutic strategy. However, the HIV-1 can escape the immune response by mutating the epitope recognized by the TCR. In this study, we re-engineered T cells by transfection of two different TCR specific for HIV-epitopes presented on different HLA-molecules simultaneously. The presence of the dual specific slow down the process. CD8+ T cells transfected with a Gag- and a Non-specific TCR released cytokines (IL-10, TNF, and IL-8) after antigen specific stimulation. However, cytokine secretion from specific TCRs were inhibited by competitive effects between both TCR. To prevent mis-pairing and resulting competitive effects, the constant region of the Non-TCR were exchanged with mutant constant regions. The maturation resulted in a higher expression and cytokine secretion when the Non-TCR was introduced alone in the T cells, however, had no influence on the competitive effect in the dual-specific T cells. Cells reprogrammed with the mismatched TCR, either introduced alone or in combination with the Gag-TCR, led antigen-loaded target cells more effectively than T cells transfected with the human TCR. Interestingly, after antigen-specific stimulation of one of the TCRs on dual-specific T cells, this TCR was functionally down-regulated, without influencing the surface expression of the second introduced TCR. Furthermore, we were able to prove that both TCRs were efficiently transfected into the same CD8+ T cells. Taken together, CD8+ T cells, which were transfected with two different TCRs can represent a new tool to study TCR functionality and might be used in the adoptive immunotherapy of HIV-infected patients.

P140 Mast cells control cutaneous lymphocytic choriomeningitis virus infection in mice

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Abstracts

Taken together, this study shows a direct comparison of CAR with different scFv specific for the same target specificity.

P141 Impaired T cell function in patients with chronic mucocutaneous candidiasis is independent from autoantibodies

H. Hofbauer,1, A. K. E. Eberhard,2, W. D. Perk1,2, T. Furtado1,2, T. T. Schröder1,2, C. Schmidt-Voß1,2, and T. Allard1,2
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Abstracts

Taken together, this study shows a direct comparison of CAR with different scFv specific for the same target specificity.
For serum dilution 1:2, 10 of 15 patients (66.7%) were PV positive in PVP expressing IgG anti-Dsg3 Abs, whereas five of 20 patients (25%) of the control group showed positive OD values (p = 0.03, Chi-test). The specificity of IgG anti-Dsg3 Abs was 88.9%, whereas for patients with PV compared to the control group. Therefore, the negative predictive value of IgG anti-Dsg3 Abs was determined for each serum dilution based on the largest Youden index (sensitivity + specificity-1). The specificity was 79% and the negative predictive value was 78.9%. The positive predictive value was 68.8%.

Conclusion: In our investigations we could demonstrate significantly higher levels of IgG anti-Dsg3 Abs by ELISA in serum dilutions 1:2 and 1:100 compared to the control group (p = 0.03). Sensitivity and specificity were both found to be high enough to apply this assay as a diagnostic tool for patients with PV.

P145 (V4) Route of T cell administration determines treat efficacy in Th1 cell based immunotherapy

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Abstracts
of DNA on melanoma cells in our model, we established the Hcscm84 cell line from a primary high-Cdkk2/14 melanoma. Hcscm84 grows in vitro with a dominant, heavily phagocytosed, and in vivo with minimal melanoma, with little immune infiltration. These tumors morphologically resemble primary melanomas in vivo.

Quantitative RT-PCR analyses show expression of the cytokine-mediating receptors R1 and MD6 as well as high levels of IL-17 in the tumor microenvironment, which we were able to confirm with flow cytometry. IL-17 production was strongly induced by the tumor-infiltrating lymphocytes (TIL) with polyethyleneimine (PEI) targets cytokine MD6, leading to secretion of the interferon-regulated chemokines CXCL10 and IFN-α and indicating transduction of endosomal LTR with naked PEI that affects these effects on tumor cells.

Based on these data, we reasoned that therapeutic targeting of NAC in vivo should promote melanoma cell death, support recruitment of cytotoxic T-cells in the tumor microenvironment and enhance melanoma cell recognition. Indeed, intratumoral injection of pol (C) complexed with PEI augments adoptive T-cell therapy in mice bearing established Hcscm84 melanomas and enables complete regression of tumors in a significant proportion of mice. Because of the slow growth kinetics of Hcscm84, we could assess the kinetics of the T-cell response and the generation and persistence of memory T-cells. Surprisingly, administration of pol (C) complexed with PEI does not affect the proliferation of adoptively transferred TcR T-cells, the acquisition of effector functions like interferon gamma production or the generation of memory T-cells, which is observed for the classical TLR ligand.

Taken together, our data indicate that stimulation of cytokine pattern recognition receptors augments the adoptive T-cell therapy of melanoma through direct effects on tumor cells in vivo.

P148 New lessons learned from cancer immunotherapy: tumor-specific CD4+ T cells frequently expand and react to short peptides

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The old paradigm says – short tumor peptides bind to MHC class I and induce CD8+ cytotoxic T-cell (CTL) responses, whereas longer peptides are required to activate CD4+ helper T cells (TH) via MHC class II restriction. However, our studies showed that both MHC class I restricted and MHC class II restricted CD8+ and CD4+ T cells with the potential to elicit autoimmunity were focused on the detection of the desired CD4+ CTL. Here we show that this is not all that tumor cells can elicit.

Andarding T cell responses against short tumor peptides derived from different tumor antigens (Mage, Melaka, Survivin) we did not only find the expected CD8+ CTL in our melanoma patients, but also frequently short peptides (11-7085) responses were largely induced in our patients. These responses were potent and tumor-inhibiting, strongly, not only against melanoma, but also against the induction of pro-inflammatory mediators such as TNF in DTHR. Importantly, TNF enhances the detection of MMP-activity by a MMP activatable OI-probe might be an applicable tool to monitor MMP-activity and angiogenesis in vivo. Thus, NF-kB dependency. In many patients those class II restricted CD4+ TH responses were up to 10 fold stronger than expected CD8+ CTL responses and indolled cells capable of producing large amounts of IFN, TNF alpha and IL2 mostly in a polynuclear manner to stimulation with 9mer peptides.

In aggregate we predicted evidence for a frequently overlooked MHC class II restricted CD4+ TH response to small tumor peptides which role in cancer immunity requires further attention.

P149 NF-κB inhibiting N-Acetylcysteine (NAC) protects from acute and chronic DTHR

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In vivo analysis of MMP-activity in vivo to the first ear challenge. We analyzed ear swelling responses 12–24 h after ear challenge and investigated BAY 11-7085 suppresses only the canonical NF-κB pathway we could detect only faint therapeutic effects. As BAY 72076 Tuebingen, Germany

Importantly, TNF enhances the induction of pro-inflammatory mediators such as TNF in DTHR. The old paradigm says – short tumor peptides bind to MHC class I and induce CD8+ cytotoxic T-cell (CTL) responses, whereas longer peptides are required to activate CD4+ helper T cells (TH) via MHC class II restriction. However, our studies showed that both MHC class I restricted and MHC class II restricted CD8+ and CD4+ T cells with the potential to elicit autoimmunity were focused on the detection of the desired CD4+ CTL. Here we show that this is not all that tumor cells can elicit.

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In aggregate we predicted evidence for a frequently overlooked MHC class II restricted CD4+ TH response to small tumor peptides which role in cancer immunity requires further attention.

P150 P150 (V01) T-bet+ Th17 cells develop in the absence of TGF-beta signaling

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In aggregate we predicted evidence for a frequently overlooked MHC class II restricted CD4+ TH response to small tumor peptides which role in cancer immunity requires further attention.

P152 Induction of anti-tumor responses against malignant melanoma via antigen targeting in vivo

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In aggregate we predicted evidence for a frequently overlooked MHC class II restricted CD4+ TH response to small tumor peptides which role in cancer immunity requires further attention.
PS14 (V14)  
The role of regulatory T cells in an HLA-class II transgenic mouse model of pemphigus vulgaris.  
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Reactive cutaneous T-cell lymphoma (CTCL) is characterized by the infiltrating skin cells, which are associated with significant morbidity and mortality.  
Aim: We investigated the effects of systemic therapy on the cardiovascular risk of psoriasis patients.  
Methods: A detailed history and laboratory analysis did not reveal any evidence of hematologic malignancies or antibiotic treatment that had occurred approximately 7 weeks before the onset of other skin symptoms.  
Conclusion: We were able to demonstrate through microdialysis a shift in the micromilieu of psoriatic plaques and possibly psoriatic skin in general and insulin resistance in the skin compartment in particular.
P616 Susceptibility of pathogenic and commensal Staphylococci to skin-derived antimicrobial proteins

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Homo sapiens releases several antimicrobial proteins (AMP) which contribute to protect the skin against infection. Staphylococcus aureus (S. aureus) represents an important pathogenic gram-positive bacterium associated with several skin infections. Recent work has shown that S. aureus human beta-defensin (HBD-3) and RNase 7 help to control skin colonization with S. aureus. Other Staphylococci such as S. epidermidis are part of the commensal skin flora. However, the role of AMP in regula-
ting the commensal flora is still emerging. Aim of this study was to gain more insight into the capacity of AMP to control the growth of commensal bacteria. Therefore we performed a system-
atic comparison of the susceptibilities of S. aureus aureus and various commensal Staphylococci to the important skin-derived AMP human beta-defensin (HBD-3) and RNase 7 and positive controls (S. aureus). We found that both AMPs were highly effective in killing commensal strains of S. lophogriseus, S. sorgenii, S. homaei, S. coebii and S. haemolyticus. HBD-3 exhibited a very low activity against S. epidermidis. Forsetin, the most abundant AMP on the skin sur-
face, barely killed S. epidermidis. Other commensal Staphylococci as well as S. aureus were also affected only at high concentrations of peptide.

In summary, our data revealed that HBD-3, HBD-7 and RNase 7 are active against S. aureus as well as against various commensal Staphylococci suggesting that these AMP may limit the growth of patho-
genic bacteria such as S. aureus which are establishing the commensal Staphylococci flora. It remains to be determined whether the weak activity of RNase 7 against S. sorgenii may explain the abundance of S. sorgenii on human skin. The low AMP activity against S. epidermidis is inline with recent data describing forsetin as an AMP with preferential antibacterial activity against E. coli. How-
ever, since forsetin is the most abundant AMP on skin surface and may act in synergy with other AMP further studies have to verify the hypothesis that forsetin has no major function in controlling the growth of Staphylococci.
Furthermore the lytic activity correlated positively with certain cytokine profiles with a pronounced correlation between lytic activity and antigen-specificity (MHC tetramer positivity) was found. Intracellular cytokine production (interferon-γ) of percentage of peptide-specific T cells, determined by MHC tetramer binding, was performed with the following assays: limiting-dilution based approach. Frozen aliquots of restimulation to specific T cell clones we chose a limiting-dilution based approach. Frozen aliquots of functional capacity.

T cell responses in melanoma patients vaccinated with autologous monocyte-derived dendritic cells (DC) loaded with peptides from different tumor-associated antigens (TAA) were characterized for their functional capacity. TAA-specific T cells were stained with melanoma specific markers. With our Cell sorter (BD ARIA) we therefore set out to develop a suitable flow cytometry procedure. As hypotheses not only detection but also isolation of the cells is necessary.

Intracellular cytokine production (interferon-γ) of percentage of peptide-specific T cells, determined by MHC tetramer binding, was performed with the following assays: limiting-dilution based approach. Frozen aliquots of restimulation to specific T cell clones we chose a limiting-dilution based approach. Frozen aliquots of functional capacity.

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Introduction: *Alternaria* species is one of the most important pathogens of nosocomial infections and a frequent opportunistic pathogen in immunocompromised hosts. Until now, genetic strain typing of *Alternaria* species could not be identified using morphological criteria. ITS sequencing revealed *Alternaria* infection for proving similarity of isolates is very suitable for comparing different strains. A useful method for characterization of the cultivated commercial vaccine strain (Bovilis thallus) appear red greyish-white with submerge growth and a verrucous surface. The microscopic picture is suitable for identifying the respective IC60 concentrations of the antiseptics tested were determined. Subsequently, the microorganisms were repeatedly inoculated with these IC60 concentrations for 100 days. Influence of the continued treatment was determined by calculation of the final IC60.

Results: A fast and dramatic increase in the IC50 of mupirocin was observed while the antiseptics showed a much lower potency to induce adaptation in *Staphylococcus aureus*. In the present study the IC60 concentration for silver nitrate was found to increase with repeated treatment of *Staphylococcus aureus*.

Conclusions: Increasing use of antiseptics may result in bacteria that are less susceptible. As wound dressings with antiseptics are more and more utilized in the treatment of critical colonized or infected chronic wounds, it is of interest to determine the risk of triggering formation of resistant bacteria. Employing microplate-laser-nephelometry it could be shown that commonly used antiseptics have a low potency to induce adaptation in *Staphylococcus aureus*. This study may help to improve the therapeutic use of antiseptics.

P176

Antigen-loaded skin migratory Langerin+ DC induce regulatory T cells during Leishmania major infection

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Abstracts

*Leishmania* infection is based on Th1/Th1c immunity, since IFN-gamma secretion of both CD4+ and CD8+ T cells plays a key role in parasite clearance. After 48 h, the supernatant was analysed for release of IFN-gamma and IL-4. The majority of induced CD8+ T cells to secrete high amounts of IFN-gamma and low levels of IL-4. The majority of the DC were isolated from lymph nodes of Langerin-DTR mice either treated with DT (Lang-DTR) or non-treated (PBS) for 9 days. Leishmania major-infected Langerin-DTR mouse tissue, where high IFN-gamma levels were found, was used as antigen for primary stimulation of HIV-infected whole cell mass spectra of reference isolates grown on a variety of solid media and at particular medium demands, and handling requirements of individual isolates. Another strategy is to store and sample isolates. This can, however, be rather impractical due to differences in growth behaviour, and sample isolates. This can, however, be rather impractical due to differences in growth behaviour, and sample isolates. The identification of microorganisms by MALDI-TOF MS is about to replace biochemical identification. However, in contrast to biochemical identification, the identification by MALDI-TOF MS is only based on a comparison of spectra of unknown isolates with spectra of known reference isolates. In clinical practice, MALDI-TOF MS has already been shown to be of great diagnostic value for the identification of microorganisms, especially in the field of dermatological infections. The identification of microorganisms by MALDI-TOF MS is only based on a comparison of spectra of unknown isolates with spectra of known reference isolates. In clinical practice, MALDI-TOF MS has already been shown to be of great diagnostic value for the identification of microorganisms, especially in the field of dermatological infections.

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P175

A PBMC-transfer model to analyze human cutaneous leishmaniasis – suitability analysis of various immunomoductive mouse 

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Abstracts

*Leishmania* species are used as recipients of human peripheral lymph mononuclear cells (PBMC) for in vitro assays of human immune functions. In the present study, we intended to establish a human tissue model for cutaneous leishmaniasis (CL). To this end, PBMC from immunodeficient mouse strains NOD-Scid, NOD-Scid gamma-(−/−), NOD-Scid-sg5 (H-2Bi, A-2Bi, R-2Bi, B-2Bi)-SCID mice and non-human primates were used as healthy volunteers. This was followed by intradermal injection with 1000 microliters each parenteral Gm2 ganglioside (H-2Bi, A-2Bi, R-2Bi, B-2Bi)-SCID mice and non-human primates were used as healthy volunteers. This was followed by intradermal injection with 1000 microliters each parenteral Gm2 ganglioside. In summary, identification of novel CD8+ (and CD4+) T cell epitopes would (a) allow for detailed analysis of T cell development in infections with the parasite using immunomonitoring and immunotechnology and (b) aid the development of a vaccine against this important human pathogen.

P178

P178

Characterization of Leishmania-derived CD8+ T cell epitopes by a combination of proteome analysis, epitope prediction followed by in vitro and subsequent in vivo analysis

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Abstracts

Cutaneous infections with *Alternaria* species are rare and mostly diagnosed in immuno-compromised patients. Until now, genetic strain typing of *Alternaria* species could not be identified using morphological criteria. ITS sequencing revealed *Alternaria* infection for proving similarity of isolates is very suitable for comparing different strains. A useful method for characterization of the cultivated commercial vaccine strain (Bovilis thallus) appear red greyish-white with submerge growth and a verrucous surface. The microscopic picture is suitable for identifying the respective IC60 concentrations of the antiseptics tested were determined. Subsequently, the microorganisms were repeatedly inoculated with these IC60 concentrations for 100 days. Influence of the continued treatment was determined by calculation of the final IC60.

Results: A fast and dramatic increase in the IC50 of mupirocin was observed while the antiseptics showed a much lower potency to induce adaptation in *Staphylococcus aureus*. In the present study the IC60 concentration for silver nitrate was found to increase with repeated treatment of *Staphylococcus aureus*.
different infection times. Generally, reference isolates are inoculated on three different media and multiple plates for each of the three different infection times. By this way 3 x 3 approach the variability of mass fingerprints of individual isolates is largely captured and the mass spectra we deposed in the reference database. When multiple isolates of a species are contained in the database, the corresponding data was used to compute superfluffers for fully automated identification. FARSAMIS allows the rapid, automated identification of most clinically relevant fungi by direct on-target matrix-assisted laser desorption ionization. Integration of polymorphonuclear leukocytes (PMN)-mediated upregulation of epithelial TLR4 and concomitant protection against fungal infection, which is independent of PMN-polymorphonuclear cell contact. Candida invasion and cell injury could be reduced by blocking TLR4 signaling using antibiotics or RNA interference. Antibody neutralization studies demonstrated that the TLR4-mediated upregulation of epithelial TLR4 is required for the additional PMN response in the C. albicans infected and H2O2 model, not only to upregulate epithelial TLR4 expression but also to modulate a broad array of inflammatory mediator release from PMN and epithelial cells might play a crucial role in the protective effect against C. albicans, which is not mediated by the TLR9-mediated PMN-mediated upregulated TLR4 expression. We confirmed the protective role of LL-37 by exogenous addition, which reduced the secretion of inflammatory cytokines and TLR4 'knockdown' (RNAi), demonstrating the direct role of LL-37 in the protection from C. albicans-mediated epithelial injury and pro-inflammatory cytokine induction. PMNs can be important innate immune cell, which are directly responsible for protecting the mucosal membrane of the skin from colonization with C. albicans, oral epithelium and PMNs results in the PMN-mediated upregulated expression of epithelial TLR4, which is directly responsible for protecting the mucosal surface from fungal invasion and cell injury by the secretion of LL-37 via PMNs.

**P179**

Involvement of the transcription factor aryl hydrocarbon receptor in Leishmania major infection of macrophages

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Leishmania major infection of macrophages is a crucial step in the establishment and maintenance of infection. Two established model systems are available for studying the mechanisms of innate immunity and T cell response in specific immunity

**P180**

Infection with Staphylococcus aureus is decided by both early mechanisms of innate immunity and T cell response in specific immunity


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Inflammation is decided by both early mechanisms of innate immunity and T cell response in specific immunity

**P181**

Commensal amplifies the innate immune response to pathogens in human skin

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Little is known about the impact of signals delivered by commensal on skin barrier function and the innate immune response to pathogens. We show that commensal and pathogenic staphylococci differ in their ability to induce expression of antimicrobial peptide/14 genes in different commensal pathogeneity in human primary keratinocytes. Whereas secreted factors of S. epidermidis induce expression of the AMP-1, HBD-3 and RNase A in primary human keratinocytes via TLR2- and EGFR- and NFkB-activation, those of S. aureus activate the MAPK and PI3K/AKT signaling pathways and suppress NFkB activation by upregulation of IκB. Interestingly, commensal staphylococci are able to modulate the innate immune response of human keratinocytes to pathogenic bacteria by partial induction of AMP expression and abrogation of NFkB suppression suggesting that the two pathogenesis in human keratinocytes may be very different. We identified TLR2 as key regulator of TLR2-mediated innate immunity. These data indicate that S. aureus derived TLR2 ligands shift TLR2 dependent innate immune inflammation towards chronic and persistent responses through a concerted activation of TLR2 and IL-4RI.

**P182**

Induction of mouse beta-defensins in the skin of lipoxegenase (AloxO3E and AloxO12B) deficient mice with an ichthyosis-like phenotype

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Autosomal-recessive congenital ichthyosis is a heterogeneous group of hereditary keratinization disorders characterized by atrophoderma of the scrotum, disturbed epidermal differentiation, and a disturbed skin barrier function. Mutations in AloxO3E and AloxO12B genes, which code for two different epidermal lipoxigenases preferentially expressed in the skin, were found in patients with ichthyosis vulgaris. We hypothesized that different courses of disease in patients with AloxO3E and AloxO12B deficiency are caused by different interactions of these enzymes with skin pathogen and by different functions of murine skin-derived keratinocytes. As mice with mutations in the AloxO3E and AloxO12B genes show an ichthyosis-like phenotype with an impairment of skin barrier function, we asked whether the beta-defensin is induced in these mice. Flank skin samples from AloxO3E and AloxO12B deficient mice were analyzed for expression of mouse beta-defensin-1, -3, -5, -14 and -20 and protein expression of beta-defensin-14 as measured by Western blot in skin samples from mice with mutations in AloxO3E and AloxO12B deficient mice. The increase in beta-defensin expression may participate in the loss of skin barrier function in mice with an ichthyosis-like phenotype.

**P183**

Mucin skin infection with Staphylococcus aureus is decided by both early mechanisms of innate immunity and T cell response in specific immunity


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Skin infection with Staphylococcus aureus is decided by both early mechanisms of innate immunity and T cell response in specific immunity

**P184**

Extracellular matrix components in skin barrier function

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Skin barrier function is an important parameter for cutaneous inflammation. Different components of extracellular matrix and protease inhibitors of extracellular matrix are responsible for maintaining stratum corneum hydration and barrier.

**Abstracts**

The abstracts are not part of the natural text and are included in the table of contents for reference. They provide a brief overview of the research presented in the document, highlighting key findings and methodologies. These abstracts are typically short and to the point, aiming to capture the essence of the research work. They are often used in academic conferences and publications to give a quick summary of the research without delving into the detailed findings. The abstracts serve as a valuable tool for researchers, as they can quickly identify relevant studies for their own work and for understanding the broader context of scientific inquiry in a specific field. In the context of the provided document, the abstracts likely summarize the main findings and contributions of each research study, accompanied by relevant methodologies and conclusions. These abstracts are essential for readers to gain a succinct understanding of the research without having to read the entire document. They provide a concise overview of the research, enabling readers to efficiently assimilate and reference the key points. The abstracts can be particularly useful for those who need to quickly assess the relevance of a study to their own research interests or to stay informed about the latest developments in a particular scientific area.
Lactobacillus are a family of heterotrophic extracellular matrix glycoproteins in the basement membrane of different tissues. They are found in the skin, the gut, and the lungs, and they have various functions, including the production of extracellular matrix glycoproteins, which are known to be sensitive to proteolytic processing. Interestingly, the LRG-5 fragment has been shown to inhibit the growth of Staphylococcus aureus and to induce the production of pro-inflammatory cytokines, which are known to be involved in the pathogenesis of skin infections. In the present study, we aimed to explore the role of LRG-5 in the skin infection of AD patients, and to assess its potential as a drug target for the treatment of skin infections.

Methods: The study included 30 patients with moderate to severe AD, aged between 18 and 65 years. The patients were randomly assigned to one of the following treatment groups: (i) LRG-5 injection, (ii) LRG-5 injection plus the antibiotic ciprofloxacin, and (iii) placebo injection. The primary outcome measure was the change in the severity of skin lesions, as assessed by the SCORAD index, after 8 weeks of treatment. The secondary outcome measures included the change in the levels of pro-inflammatory cytokines, as assessed by ELISA.

Results: The SCORAD index decreased significantly more in the LRG-5 and LRG-5+ciprofloxacin groups compared to the placebo group (p<0.05). The levels of pro-inflammatory cytokines also decreased significantly more in the LRG-5 and LRG-5+ciprofloxacin groups compared to the placebo group (p<0.05).

Conclusion: LRG-5 injection is a promising treatment for skin infections in AD patients, and it may be used as a drug target for the treatment of skin infections.
Role of dipeptidyl peptidase IV and related enzymes in the regulation of DNA synthesis of skin cells

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Dipeptidyl peptidase IV (DPP IV) is an ectoenzyme up-regulated in proliferative skin diseases like psoriasis. Primary human keratinocytes, skin fibroblasts and sebocytes were previously described to express DPP IV/CDC26 on the cell surface. The non-selective inhibition of DPP IV activity, Lexi(ZIN201), shamtide (ZIN206) and prelydian (ZIN207) suppress proliferation and cytotoxic production of these cells in vitro, suggesting this enzyme as a target for drug therapy of skin diseases like eczema or psoriasis. The role of other DPP IV-related enzymes like DPP 8/9 in the regulation of cellular functions was hypothesised, because sitagliptin, a D P IV-selective inhibitor approved for diabetes therapy, lacks immunosuppressive activity. The aim of the present investigation was to clarify the role of D P IV activity and D P IV-related enzymes in the regulation of DNA synthesis of skin cells.

We studied expression of D P IV in relation to D P IV by quantitative RT-PCR and investigated the dose- and time-dependent maximum suppression of Pro-Pro-pNA hydrolysis and [3H]-Thymidine incorporation in the presence of a D P IV inhibitor, which has no effect on DNA synthesis. Moreover, we observed that in NIH 3T3 keratinocytes, D P IV-IV suppresses 20.6% of enzyme activity and 53.8% of DNA synthesis, whereas neither enzyme activity nor DNA synthesis are reduced. In D 293 cells, the DP IV-selective inhibitor has minor effects on enzyme activity (4.8%) and DNA synthesis (4.1%). A combination of a D P IV and a DP 8/9-specific inhibitor shows additive effects on suppression of enzyme activity, but no similar effect on proliferation, and is comparable to the effects seen with ZIN206. Prelydian inhibits the non-selective drugs for diabetes therapy. The lack of their effects on the skin cells were achieved by those inhibitors that bind to DP IV, but not at the active site. Our data suggest that the suppression of cell proliferation, the inhibitory capacity toward DP IV activity or distinct purified DP IV enzymes is not crucial. With respect to immunohistochemical data for DP IV, it is a highly probable that nontarget enzymes are in the access of natural substances and/or the induction of counterterrestrial enzyme structure changes are much more relevant. The most probable explanation is an alternative binding site at DP IV mediating the antiproliferative. These data support the novel model of cellular DP IV function developed recently by IMTM.
To further characterize this phenomenon, thrombomodulin (CD142) and BAIAP2 mice were exposed to UVB (80 mJ/cm²) 14 days before sacrifice. The analysis of skin sections 0–24 h later showed a dose- and time-dependent but still incomplete manifestation of Purpura solares. Moreover, UVB irradiation induced a significant cutaneous influx of neutrophils that was observed on histological sections and quantified by mean activity of neutrophil-specific myeloperoxidase (MPO). The UVB-induced skin bleeding is strictly limited to the area of exposure and can be prevented by dose-reduction e.g. via topical application of sunscreen (SPF > 50).

As UV-irradiation induces a rapid influx of leukocytes, their role was further investigated in UVB-induced skin hemorrhage under thrombocytopenic conditions. To this end, mice were treated with a neutrophil depleting antibody prior to UVB-irradiation. Interestingly, under thrombocytopenic conditions skin bleeding was virtually ablated. These findings suggest that leukocyte extravasation is required in the development of Purpura solares. The role of chemokinent stimuli essential for leukocyte recruitment is matter of current investigation.

In summary, during the absence of plaques UVB irradiation induces Purpura solares which can be prevented by inhibiting cutaneous leukocyte recruitment.

P198
Angiopoietin-2 stimulation induces tk3 integrin internalization and degradation
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Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2) have been identified as agonist and antagonist of the endothelial tyrosine kinase receptor Tie2. While Ang-1 induces phosphorylation and stabilizes endothelial cell survival, Ang-2 interferes negatively and induces endothelial destabilization. Both, Ang-1 and Ang-2, have been determined to induce transcriptional up-regulation of its receptor Tie2. To molecularly decipher the mechanisms of Ang-1-mediated endothelial cell destabilization, we examined Tie2 mRNA and protein expression, degradation, internalization and subsequent endothelial cell survival. Ang-2 interferes negatively and induces endothelial destabilization. While Ang-1 and Ang-2 have been demonstrated to induce transcriptional up-regulation of its receptor Tie2. To molecularly decipher the mechanisms of Ang-1-mediated endothelial cell destabilization, we examined Tie2 mRNA and protein expression, degradation, internalization and subsequent endothelial cell survival. Ang-2 interferes negatively and induces endothelial destabilization.

Together, these findings suggest that the activation of the PI3K-AKT signalling pathway is relevant for activated AKT, whereas the surrounding tumor-free brain tissue was negative for activated AKT. A histochemical analysis showed that melanoma brain metastases of 10 patients were highly positive for activated AKT, whereas the surrounding tumor-free brain tissue was negative for activated AKT. Therefore, new therapy strategies are mandatory.

The efficacy of temozolomide in melanoma treatment is low (response rate <20%) and may depend on the activity of other cellular defense mechanisms. Further, melphalan (L-2), but not cisplatin (P-2), significantly increased sensitivity to a prolonged, whereas MelA, MelB, and MelC cells were surprisingly more sensitive to a short treatment with temozolomide. The results indicate that a prolonged, whereas MelA, MelB, and MelC cells were surprisingly more sensitive to a short treatment with temozolomide. Therefore, new therapy strategies are mandatory.

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TRAIL-induced apoptosis. In conclusion, the study provides a rationale for the use of NS2A as a potentially new therapeutic option for cutaneous T-cell lymphoma.

P206 (V21)

Melanoma cells control synthesis of hyaluronic acid in peritumoral fibroblasts via TGFbeta, PDGF-AA and PDGF-CC: impact on melanoma cell proliferation

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Abstract: TGF-β, PDGF and hyaluronic acid (HA) play a crucial role in tumor progression. Stromal-derived HA may support tumor cell proliferation and migration. Previously we have shown that soluble factors from tumor cells of Malignant Melanoma (MM) elevate the synthesis of HA in stromal fibroblasts through the induction of HA-Synthase (HAS) 1 and 2. Based on these findings we aimed to identify the functional interactions between the fibroblasts and the cells and the soluble factors that are involved in the stromal-tumor-stroma interaction.

Using co-culture experiments we could demonstrate that MM cell lines B16 and HT14 show increased cell proliferation when grown on a layer of HA-secretting fibroblasts. Blocking of HA-synthesis in fibroblasts with 4-Nitro-2-imidazolone abrogated this effect. Melanoma cell line conditioned medium (MMCM) potently induced HAS1 and HAS2 mRNA and HA-synthetase in fibroblasts indicating that soluble factors released by melanoma cells are responsible for this effect. To identify the MM-derived mediators and their respective receptors on fibroblasts stimulations with recombinant factors, sFNA transactions and function-blocking antibodies were used. The involvement of MM-derived metabolites (lactate) and several cytokines like IL-6 or IFNβ could be excluded.

We could show that TGF-β is the stimulating mediator for HAS1 and sHGFβ2 in Hce cells abrogated this induction. The growth factors PDGFA-A and PDGFC-C induce HAS2 expression in baf-b cells. Furthermore, sHGFα and PDGFC-C mRNA in melanoma cells and/or blocking PDGFR-B on fibroblasts could reduce the observed stimulation by MMCM.

Taken together, melanoma-derived mediators TGFβ, PDGFC-A and PDGFC-C stimulate HA-synthase in stromal fibroblasts thus enhancing melanoma cell proliferation.

P207

Cell adhesion dependent regulation of the transcription factor c-Jun in melanoma

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The transcription factor c-Jun is a key player in the process of cell proliferation, apoptosis and differen-
tiation in tumor progression. It forms dimers with other members of the transcription factor super-
family AP-1, influencing the expression of a multitude of genes involved in tumor development and metas-
	asis. However, the role of c-Jun in melanoma is largely unknown. Here we investigated the role of c-Jun in the melanoma cell line A375.

We revealed that loss of E-cadherin during development of melanoma leads to induction of c-Jun pro-
tein expression. Interestingly, the mRNA level of c-Jun protein was not affected, suggesting that c-Jun is regu-
lated on posttranscriptional level in melanoma. Here, we present data that the dynamic cytoskeletal mu-

tpetition of c-Jun is involved in the transcription of c-Jun protein. Immunofluorescence experiments with cytoskeletal disrupting agents taxol and nocodazole hint for cytoskeletal dependent regulation of c-Jun protein.

In conclusion, the findings strongly suggest that the cytoskeleton regulates the transcriptional activ-
ity of c-Jun which we could recently substantiate by demonstrating oncogene addiction of MCC to the MCV T-
antigen (T)."
P214
Melanoma-derived VEGF triggers an acute endothelial cell activation
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Abstracts


P215
Autoantibodies against CD28 - a new prognostic marker in malignant melanoma with impact on therapies with interferons?
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Abstracts

Results: Sixteen of 101 (15.84%) patients with melanoma showed CD28-abs, whereas their prevalence among 152 patients with hay fever or asthma donors were investigated. Serum samples from 101 patients with malignant melanoma, 152 patients with hay fever or asthma, and 152 healthy donors were investigated.

Conclusion: CD28-abs are a frequent finding in malignant melanoma patients. In contrast to the findings in patients with asthma or hay fever, no significant correlation of CD28-abs and RAST reaction was found. This indicates that CD28-abs are stronger associated with malignant melanoma than with atopic diseases. The occurrence and impact of CD28-abs in malignant melanoma and other neo- plasms has not yet been investigated.

P216
Differential expression of ZO-1 in melanoma and nevi and their tumor microenvironment – an indicator of malignancy
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Abstracts

Conclusions: Further observation of the clinical course is needed to investigate whether the presence of CD28-abs is associated with a shortened progression-free and overall survival or not. Interferons seem to induce the production of CD28-abs. It cannot be ruled out that CD28-abs stimulate regulatory T-cells and lead to suppression of T-cell responses resulting in immunosuppression in melanoma patients. It is discussed that CD28-abs may occur in patients receiving interferons. In vitro cell models may further elucidate the role of CD28-abs in melanoma.

P217
Modulation of NOXA and MCL-1 as a tactic for sensitizing melanoma cells to the BH3-mimetic ABT-737
N. Mohana-Kumaran1, K. Lucas1, W. Weninger1, T. R. Commis1, A.糖状の膜は、毛細血管の侵襲をもたらすと解釈される。我々は、この現象は、細胞が、血管の内皮細胞に存在するZO-1を認識して、血管の壁に付着するようにする。Vegfの過剰発現を抑制することで、これらの細胞は、血管の壁に付着し、治療の対象となる。

Conclusions:

P215
Autoantibodies against CD28 - a new prognostic marker in malignant melanoma with impact on therapies with interferons?
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Abstracts

Results: Sixteen of 101 (15.84%) patients with melanoma showed CD28-abs, whereas their prevalence among 152 patients with hay fever or asthma donors were investigated. Serum samples from 101 patients with malignant melanoma, 152 patients with hay fever or asthma, and 152 healthy donors were investigated.

Conclusion: CD28-abs are a frequent finding in malignant melanoma patients. In contrast to the findings in patients with asthma or hay fever, no significant correlation of CD28-abs and RAST reaction was found. This indicates that CD28-abs are stronger associated with malignant melanoma than with atopic diseases. The occurrence and impact of CD28-abs in malignant melanoma and other neoplasms has not yet been investigated.
Regulation of proliferative activity and proinflammatory chemokine expression in Hgf-Cdk4R24C mouse melanoma cell lines


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Introduction: KIT is a receptor tyrosine kinase important for growth and survival functions. Kit+ melanoma cells are known to present activating KIT gene mutations (exon 11 or 17) in almost all stages of melanoma. KIT mutations are present in 15–20% of acro lentiginous (ALM) and acral melanomas (MM). Kit is a target of the kinase inhibitor Imatinib. This drug was successfully used in Kit+ melanoma metastases in the clinic.

Objective: To investigate the expression of chemokines in melanoma cell lines along with Kit activation and the impact of Imatinib.

Methods: Cell lines were generated by transduction of melanoma cell lines with lentiviral constructs presenting either constitutively active Kit (V560E) or Kit (del709) and were selected for drug resistance. Expression of Kit and its phosphorylation state were determined by flow cytometry and Western blotting. Kit phosphorylation was also confirmed by mass spectrometry. RNA expression of Kit and other transcripts was measured by qRT-PCR.

Results: Upon activation, Kit promotes melanoma cell proliferation. In response to Kit activation, melanoma cells express various chemokines. Expressions were confirmed by ELISA. Imatinib inhibits Kit signaling and reduces melanoma cell proliferation. Consistent with these results, Imatinib also reduces the expression of various chemokines. To further understand the role of chemokines in melanoma cell biology, we transduced melanoma cell lines with lentiviral constructs expressing either GROα or IP10. The chemokine GROα promotes melanoma cell proliferation, while IP10 reduces melanoma cell proliferation. The expression of GROα and IP10 was confirmed by qRT-PCR and ELISA.

Conclusion: Our study suggests that the expression of chemokines in melanoma cells is regulated by Kit activation and that Imatinib inhibits Kit signaling and reduces melanoma cell proliferation. This finding has therapeutic implications for the treatment of melanoma.

Discrepancy between Kit+ mutations in vivo and in vitro in acro lentiginous and mucosal melanoma


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Introduction: KIT is a receptor tyrosine kinase important for growth and survival functions. cKIT mutations are present in 15–20% of acro lentiginous (ALM) and acral melanomas (MM). cKIT is a target of the kinase inhibitor Imatinib. This drug was successfully used in cKIT+ melanoma metastases in the clinic.

Objective: To investigate the expression of cKIT in melanoma cell lines along with cKIT activation and the impact of Imatinib.

Methods: Cell lines were generated by transduction of melanoma cell lines with lentiviral constructs presenting either constitutively active cKIT (V560E) or cKIT (del709) and were selected for drug resistance. Expression of cKIT and its phosphorylation state were determined by flow cytometry and Western blotting. cKIT phosphorylation was also confirmed by mass spectrometry. RNA expression of cKIT and other transcripts was measured by qRT-PCR.

Results: Upon activation, cKIT promotes melanoma cell proliferation. In response to cKIT activation, melanoma cells express various chemokines. Expressions were confirmed by ELISA. Imatinib inhibits cKIT signaling and reduces melanoma cell proliferation. Consistent with these results, Imatinib also reduces the expression of various chemokines. To further understand the role of chemokines in melanoma cell biology, we transduced melanoma cell lines with lentiviral constructs expressing either GROα or IP10. The chemokine GROα promotes melanoma cell proliferation, while IP10 reduces melanoma cell proliferation. The expression of GROα and IP10 was confirmed by qRT-PCR and ELISA.

Conclusion: Our study suggests that the expression of chemokines in melanoma cells is regulated by cKIT activation and that Imatinib inhibits cKIT signaling and reduces melanoma cell proliferation. This finding has therapeutic implications for the treatment of melanoma.
SK, including multiple lesions from each patient. SK commonly harbour multiple benign oncocytic mutations in FGFR3, FGFR4, KRAS, EGFR, and AKT1, suggesting that such lesions may have arisen in a similar manner to sporadic skin cancers. Furthermore, our data provide new clues on the origin and spread of oncogenic mutations in tissues, suggesting that apparently independent (multicentric) adult benign tumours may have a clonal origin.

P230 Clues to organ-specific metastasis: liver endothelial-specific differentiation and trans-differentiation mediated by malignant melanoma and hepatocellular carcinoma

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Introduction: Mesenchymal cells of the liver are highly specific microvascular components that control blood plasma access for the liver. Upon carcinogenesis and metastasis the endothelial cells display a process of transdifferentiation that can be visualized by IHC in different expression patterns of EC and LSEC marker genes.

P229 Multiple oncogenic mutations and clonal relationship in spatially distinct benign human epidermal tumors

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Conclusion: Expression of the novel junctional protein Leda-1 was found and analysed in the B16 cell line. Homology to AJAP-1 suggests a role as a junctional modulator and potentially a promoter of invasiveness.

Furthermore, extensive proteolytic processing could be demonstrated and cleavage at a furin cleavage site was still present in endothelial cells of tumor nodules and its signal on immunohistochemistry (IHC) was largely reduced and only found in few endothelial cells. In contrast CD31 and Leda-1 expression was still present in endothelial cells of tumor nodules and its signal on immunohistochemistry (IHC) appeared to be stronger than in the surrounding healthy liver.

Conclusion: A Lesc-specific molecular signature could be identified by comparative gene expression profiling which might well contribute to the understanding of metastasis in the liver. Upon carcinogenesis and metastasis the endothelial cells displayed a process of transdifferentiation (so called capillarization) which could be visualized on IHC in different expression patterns of EC and LSEC marker genes.

P222 The dual role of IFN-α in TRAIL-induced apoptosis of melanoma cells

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Human peripheral blood leucocytes acquire the cytoplasmic molecule TRAIL-related apoptosis-inducing ligand (TRAIL) in response to IFN-α treatment. Since IFN-α is usually administered in melanoma therapy, we asked the question whether the induction of TRAIL-α cells includes a role in preventing disease progression. To this end we analysed the susceptibility of established melanoma cells lines and primary human melanoma patients to TRAIL. Over night treatment of melanoma cells with soluble TRAIL induced apoptosis ranging from 6% to 54%, as determined by Annexin V staining. Since TRAIL acts via TRAIL receptors (TRAIL-R) we analysed the TRAIL-R expression pattern on melanoma cells by flow cytometry. We generally detected moderate to high levels of the TRAIL-R1, TRAIL-R2 and TRAIL-R3, whereas TRAIL-R4, the third receptor, was not observed on any melanoma cell line. TRAIL-R1 and TRAIL-R2 expression were reduced in the TRAIL-R2 mRNA levels, which were generally 2-3 fold lower in the melanoma cell lines than in the respective nontransformed melanocytes. Since TRAIL-induced apoptosis was not correlated with TRAIL-R1 and TRAIL-R2 expression, we investigated if the TRAIL-R3 and TRAIL-R4 expression levels were decreased under conditions of high cellular proliferation. Treatment of melanoma cells with IFN-α alone inhibited proliferation but did not induce apoptosis. IFN-α pre-treatment with TRAIL induced apoptosis, suggesting effects on TRAIL-R expression or other mechanisms of the differential response. Our findings suggest that the capacity to TRAIL-induced apoptosis is a hallmark of melanoma cells with high proliferation rates.

P227 Tumor-specific T helper 1 (Th1) cells prevent phenotypical transformation of epithelial cells into cancer in vivo

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Within 12 weeks, cancers of sham-treated mice did not only grow 10-times faster, they also displayed an aggressive phenotype. In sharp contrast, IFN-γ did not induce any sign of apoptotic damage of the nuclei or cytoplasm in vitro (apoptotic cells remained £ 5%). Double-staining with SA-β-gal/synaptophysin revealed that the senescent phenotype occurred in synaptophysin+ cancer cells. Together, the data show that specific Th1 cells directed against a tumor-associated antigen arrest tumor development by IFN-γ-mediated inhibition of cell proliferation, in the absence of major cytotoxic effects. As IFN-γ prevented carcinogenesis development without any sign of apoptosis in vivo, induction of cancer cell senescence may be a central mechanism underlying the anti-tumor effects of IFN-γ-producing Th1 cells.

P228 Multiple oncogenic mutations and clonal relationship in spatially distinct benign human epidermal tumors

C. Hafrid1, A. Töll1, A. Fernández-Casado2, J. Earl3, M. Marqués3, F. Acquadro4, M. Mendoza-Pertuz3, M. C. Hafner1, A. Toll2, A. Fernández-Casado2, J. Earl3, M. Marqués3, F. Acquadro4, M. Mendoza-Pertuz3, M. C. Hafner1

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Conclusion: Expression of the novel junctional protein Leda-1 was found and analysed in the B16 cell line. Homology to AJAP-1 suggests a role as a junctional modulator and potentially a promoter of invasiveness.

Furthermore, extensive proteolytic processing could be demonstrated and cleavage at a furin cleavage site was still present in endothelial cells of tumor nodules and its signal on immunohistochemistry (IHC) appeared to be stronger than in the surrounding healthy liver.

Conclusion: A Lesc-specific molecular signature could be identified by comparative gene expression profiling which might well contribute to the understanding of metastasis in the liver. Upon carcinogenesis and metastasis the endothelial cells displayed a process of transdifferentiation (so called capillarization) which could be visualized on IHC in different expression patterns of EC and LSEC marker genes.

P232 Absence of BRAF and HRAS mutations in eruptive Spitz nevi

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Background: Spitz nevi (SN) are rare benign tumors that occur on all skin types. The Spitzoid morphology makes them clinically distinctive from more sinister melanocytic lesions. Although some SNs exhibit a higher risk of malignancy, the majority do not. A closer examination of SNs from different ethnic backgrounds reveals a higher risk of malignancy in Asian SNs. However, the role of oncogenic mutations in Spitzoid tumors is still under investigation. In previous studies we found that 14% of B16-L6 tumor cells derived from the B16-F10 melanoma cell line did not grow when co-cultured with human skin fibroblasts. We have previously shown that melanoma cells that grow in co-culture with skin fibroblasts have a higher tendency for skin fibroblasts than melanoma cells that do not grow in co-culture. This leads us to believe that the higher tendency for skin fibroblasts may be a result of the presence of oncogenic mutations in the melanoma cells. The aim of this study was to determine the frequency of oncogenic mutations in Spitzoid tumors and to compare the frequency of oncogenic mutations in Spitzoid tumors to that of other types of skin tumors.
Novel tripteronid enriched mistletoe extracts show anti cancer effects on murine B16.F10 melanomas in vivo

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Abstract

The present study investigated the anti tumoral activity of novel mistletoe extracts containing 14-3-3©-inhibitory peptides in a murine B16.F10 melanoma model. Mistletoe extracts were prepared from Arctostaphylos uva-ursi (L.) S. Wats. (Ericaceae) and were enriched with recombinant human P239, a 14-3-3© dominant negative peptide. Melanoma-bearing mice were treated with different mistletoe extracts administered by subcutaneous injection and the primary tumor growth and metastatic spread were analyzed. Relative to the untreated or control treatment, the novel tripteronid enriched mistletoe extracts showed antitumoral activity in vivo and the percentage of metastases was significantly reduced in comparison to the untreated group. In conclusion, the novel mistletoe extracts containing 14-3-3©-inhibitory peptides are promising candidates for the development of novel anti-cancer therapies.
acquired the melanoma specific molecules M-CSF or MCAM respectively, while the efficiency depends on the molecular mechanism of beta-catenin internalization and specifically the transfer of M-CSF. Furthermore, ex vivo isolated melanoma-derived CD14+ and CD4+ cells carried MCSD, but CD14- cells showed amore intense M-CSF signal. So far we demonstrated that testing, chemoresistance especially when cells were plated on Collagen I matrix. Furthermore, interaction of melanoma cell lines and reduces tumor growth

mRNAlevels in all metastatic melanoma cell lines tested. Inhibitors upregulate p8, CHOP, ATF4, ATF3 and TRB3 mRNA levels. Furthermore, the BRAFV600E gene expression profile. Microarray analysis showed upregulation of a series of genes (p8, CHOP, ATF4, ATF3 and TRB3) in human melanoma cells, but not in primary cells of the skin such as fibroblasts or melanocytes. Expression profiles were selected because the possible usefulness of this marker in melanoma, however the factors that lead to upregulation of BRN3A are not known. The hypothesis that repetitive doses of UVA may also play a role in the pathogenesis of melanoma is based on the observation that BRN3A levels are correlated to the risk of in vivo malignant melanoma in previously reported studies. Since more than two decades the importance of beta-catenin in melanoma progression is a matter of discussion. Recently, we found that the activation of the Wnt signalling pathway leads to beta-catenin and thus inducing its degradation is down-regulated in metastatic melanoma cells, suggesting an important role of beta-catenin during melanoma progression (Sinnberg et al. 2010). To analyse the impact of beta-catenin on malignant growth and invasive properties we investigated the effects of different stages including benign melanomas were assayed for their sensitivity to beta-catenin inhibition. Interestingly, we found a high level of invasive capability and induction of apoptosis in metastatic melanoma cells, whereas early growth phase melanoma cells were less affected. Primary melanomas were completely uninfected by beta-catenin inhibition. The strong suppression induction in metastatic melanoma cells indicates that beta-catenin is an essential survival factor in late-stage melanoma cells and plays an important role in melanoma progression.

Regulation of BRN3A in melanoma

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Melanoma is due to its resistance to therapy in advanced stages – the skin cancer with the highest mortality rate. We have previously shown that the neuronal transcription factor BRN3A is highly expressed in human melanoma cells, but not in primary cells of the skin such as fibroblasts or melanocytes. Expression profiles were selected because the possible usefulness of this marker in melanoma, however the factors that lead to upregulation of BRN3A are not known. To gain more insight in the role of UVA in the pathogenesis of melanoma it is important to focus investigations on model systems resembling the human skin better than single cell cultures. To address this issue, we could previously show, that repetitive exposure of melanoma cell lines to UVA irradiation increases cellular lactate and glucose levels upon completion of UVA treatment. These findings support the hypothesis that repetitive exposure of UVA may also play a role in the pathogenesis of melanoma.

Let's now take a closer look at the role of UVA in melanoma progression. While recent studies elegantly demonstrated a causal role for UVA in the pathogenesis of melanoma, we established a reporter system by which we could monitor the activity of the YB-1 promoter. Inhibition of the PI3K/AKT signaling pathway using siRNA against ERK1/2 and PI3K delta led to reduced YB-1 promoter activity, whereas inhibition of the MAPK signaling pathway had no effect. The specificity of the signal transduction inhibitors was confirmed using siRNA against Erk1/2 for the MAPK signaling and AKT3 for PI3K/AKT signaling. Furthermore, we have recently shown that repetitive exposure of YB-1 in melanoma cells is based on the observation that BRN3A levels are correlated to the risk of in vivo malignant melanoma in previously reported studies. Since more than two decades the importance of beta-catenin in melanoma progression is a matter of discussion. Recently, we found that the activation of the Wnt signalling pathway leads to beta-catenin and thus inducing its degradation is down-regulated in metastatic melanoma cells, suggesting an important role of beta-catenin during melanoma progression (Sinnberg et al. 2010). To analyse the impact of beta-catenin on malignant growth and invasive properties we investigated the effects of different stages including benign melanomas were assayed for their sensitivity to beta-catenin inhibition. Interestingly, we found a high level of invasive capability and induction of apoptosis in metastatic melanoma cells, whereas early growth phase melanoma cells were less affected. Primary melanomas were completely uninfected by beta-catenin inhibition. The strong suppression induction in metastatic melanoma cells indicates that beta-catenin is an essential survival factor in late-stage melanoma cells and plays an important role in melanoma progression.
nisms also inhibited neural crest migration (EMT) of SKMel28 melanoma cells upon transplantation in vivo, and its expression was increased in human melanoma specimens when compared to non-malignant skin. Here we show that BMPs and nodal represent highly crucial therapeutic targets for melanoma, including for drug resistance. Together, we are able to demonstrate that BMPs and nodal represent highly crucial therapeutic targets for melanoma and to induce an EMT of such pre-treated melanocytes for the first time.

**P247**

Mechanisms of cytotoxic effects of ascorbate on melanoma cells
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Despite its controversial history in cancer therapy, a large body of evidence emerged in recent years suggesting a role of ascorbic acid (vitamin C) as a modulator of tumor cell cycle, apoptosis, and angiogenesis on numerous cancer cell lines in vitro and in vivo. In clinical trials high-dose vitamin C therapy in cancer patients has been reported to reduce tumor cell viability and angiogenesis, however, clinical efficacy could only be demonstrated for a prolonged survival of stage IV cancer patients after high-dose ascorbate treatment. Here we report for the first time the vast extent of the complexity and diversity of cytotoxic mechanisms of ascorbate (vitamin C) and its metabolites (HIF-1, GLUT-1) was determined in a newly-generated tissue microarray consisting of samples of 307 melanocytic lesions accompanied by clinical follow-up data (between 10 and 17 years) of the German Melanoma Registry. Finally, the influence of BMP-2 and -nodal (both secreted by melanoma cells) on murine dendritic cells was assessed.

In summary, we highlight that BMP and nodal are crucial for melanoma cell invasiveness in vitro and in vivo. Moreover, we show for the first time that treatment with only single protein (BMP-2, BMP-7, nodal) is sufficient to confer melanoma phenotype to benign primary melanocytes in vitro and to induce an EMT of such pre-treated melanocytes in vivo.

Together, we are able to demonstrate that BMPs and nodal represent highly crucial therapeutic targets to prevent the spreading of primary melanomas.

**P248 (V27)**

**RAGE activity relates to melanoma clinical stages and progression**
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In addition to tumor-cell intrinsic mechanisms, melanoma initiation, growth and progression have been related to microenvironmental factors orchestrating tumor-stroma interaction. However, the mechanisms that sustain a tumor-promoting micro-environment remain largely elusive, especially in malignant melanoma.

We have recently demonstrated that the receptor for advanced glycation endproducts (RAGE) is central for modulating different non-melanoma skin tumor formation as well as for experimental chronic inflammation by sustaining positive signaling feed-forward loops regulating specific sets of pro-inflammatory cytokines in melanoma cells. Here, we describe that RAGE activity relates to human melanoma clinical stages and progression. Expression of melanoma growth and development regulatory genes (RAGE activity) include RAGE protein expression, serum levels of a soluble form of RAGE (sRAGE), phosphorylation of MAP kinases (p-p38 and p-ERK), activation and secretion of tumor necrosis factor and the protein expression of RAGE (tSIP) and activating ligands such as S100A8, S100B, GBP1 in human melanoma patients. As determined by microarray analyses on human melanoma cell lines, sections of RAGE protein expression is up-regulated in a stage-dependent manner; by using sRAGE specific ELISA, ELISA levels of sRAGE are significantly down-regulated in the sera of melanoma patients at stage II compared to patients at stage I and II. Moreover, sRAGE serum levels are significantly down-regulated in patients at stage IV compared to any other stage. Activity of p38, Jun and NF-κB were demonstrated to induce transcription of ZEB1, GBP1 and sRAGE on human melanoma tissues ischemic correlated concomitantly in a stage-dependent manner in these findings in humans are at least partly resembled in mice at late stage IV. Using MTOC transmigration and transplantation melanoma mouse models as well as RAGE-deficient mice.

In conclusion, we provide multiple evidence for a novel role of RAGE signaling in driving melanoma cell proliferation and development of the melanoma microenvironment. Moreover, we demonstrate that sRAGE is a new component of the tumor microenvironment and can be considered a potential target for anti-melanoma therapy.

**P249**

Slug augments the epithelial-mesenchymal like transition in melanoma through transcriptional activation of ZEB1
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Epithelial – Mesenchymal Transition (EMT) is an important step in tumour development and it is a hallmark of aggressiveness in both epithelial and mesenchymal cells. Slug andZEB1 and ZEB2 are key transcription factors that contribute to EMT. Both Slug andZEB1 have been described as mediators of cell-cell adhesion and migration, representing the adhesion of the epithelial molecule E-cadherin. Histological assessment revealed that ZEB1 is activated at the transcriptional level by Slug. Lentiviral overexpression of Slug in WM9 or WM164 melanoma cells is followed by upregulation of both, at the mRNA and protein level, whereas silencing of Slug leads to the reverse effect. Four potent target sequence (E-boxes) for Slug were identified at the ZEB1 promoter in a region from -5800 to +200 relative to the transcriptional initiation site. Gel shift assay revealed that Slug-induced binding of a DNA-protein complex at these four E-boxes with different affinities. Luciferase assays confirmed that Slug is initiating transcriptional activity at the ZEB1 promoter. The effects of Slug on ZEB1 promoter regulation are specific, since Slug is not binding to TWIST and Twist only binding with low affinity of gel shift assay, but Twist does not lead to significantly enhanced luciferase activity. Further, Slug and ZEB1 cooperatively regulate E-cadherin expression and the effect of both EMTs on cell-cell adhesion and cell migration is additive. These studies suggest that a hierarchical and cooperative sequence inactivation of EMTs result in successful changes of the epithelial phenotype in melanoma.

**P250 (V03)**

**cIAPs block TRAIL-mediated cell death by interference with RIPoptosome formation, a novel RIP/caspase-8 containing intracellular complex**
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Signals activated by ligation of innate immune receptors such as Toll-like receptors (TLRs) are of major importance for the control of bacterial, fungal and viral infections, as well as for the development and regulation of adaptive immunity. However, overactivation of these receptors is prone to pathogenesis or malignancies. Moreover, TRAIL-induced signal influences qualitative and quantitative immune responses in the skin. In this report, we show that cellular inhibitors of apoptosis proteins (c-IAPs) are transcriptional targets of TRAIL. c-IAP2 knockdown resulted in sensitization to cell death induction in cells. Using common-pathogen-precipitation under native TRAIL settings, c-IAP2 up-regulation was evident in melanoma tissues of a novel thus far unknown intracellular complex, that we designate the ‘Ripoptosome’. This intracellular complex contains c-IAP2 and TRIF and is induced by TRAIL in melanoma and B16 melanoma cells. We hypothesize that c-IAP2 and TRIF can act as inhibitors of TRAIL-like receptor 3-induced signalling pathways as exemplified by their negative regulation of TRAIL-3-induced cell death. We demonstrate that loss of c-IAPs profoundly modifies the response to the mimics of the natural TRAIL ligand double stranded RNA (poly(I:C)). Poly (I:C) induced cell death in both a caspase and RIP1-kinase-dependent manner. Loss of c-IAPs, however, favours RIP3 dependent apoptosis. Viable c-IAP2 knockdown cells were sensitized to cell death induction and caspase-8, -FLIP, FADD and RIP1. Upon TRAIL ligation, this complex is recruited to the adaptor protein for the receptor of anti-apoptotic protein (RAIP) and to induce an activating-independent TRIF (AV-I) in a stimulation-dependent manner. Interestingly the c-IAP2 CASP8 inhibitor FLIP-L and FLIP-LS conferred substantial protection from IAP antagonists or TRIF- like weak inducer of apoptosis (TRAIke)- induced degradation of c-IAPs and subsequent formation of the Ripoptosome. These data imply that c-IAPs as important components of this complex. The detected deviations of TRAIL-mediated cell death from apoptosis to a necrotic form of cell death at the Ripoptosome is blocked by c-IAPs and activated by TRIF ligation, C19, and possibly other signaling pathways may have important pathophysiological consequences during inflammatory responses in the skin. Moreover modulation of the Ripoptosome in melanoma cells might impact the tumor immune response, thereby facilitating efficient tumor elimination.

**P251**

**Antitumoral efficacy of low temperature plasma against malignant melanoma cells in vitro**
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Introduction: In the last years plasma has been demonstrated to influence cell functions (induction of cell growth, DNA damage or apoptosis, necrosis), kill various types of cancer cells (e.g. glioblastoma cells) and influence gene expression. The plasma medicine include dental applications, wound debridement, skin modeling, sterilization of medical products and implant surface biocorrosion. Here we demonstrate the potent induction of apoptosis of glioma and melanoma tumor cells without harm of non-malignant cells, plasma could play a role in the treatment of skin melanoma tumor. Therefore we investigated the potency of two different plasma sources to induce apoptosis in melanoma and additional Burkitt- lymphoma and glioblastoma cells in vitro in order to demonstrate further utilization of plasma medicine in vitro (B16). Methods: The B16 mice melanoma were irradiated with a deuterium plasma (DBD) in ambient air and an atmospheric pressure plasma jet (APP) using Argon as feeding gas. The cells were grown on micro plates and irradiated for different time intervals (30 s–2 + 2 h). After irradiation the viability, cytostasis and apoptosis were measured after 24, 48 and 72 h. Results: Ninety seconds plasma irradiation using DBD or APP pre- induced a marked induction of apoptosis in melanoma cells measurable 48 h after treatment. The viability of cells showed a decrease 72 h after irradiation. Conclusion: We conclude a treatment of at least 90 s to be necessary for in vitro treatment of malignant melanoma metastases in mice.
basal layer is abnormally expressed by suprabasal cells. Terminal differentiating cells can communicate with other cells and tumor microenvironment including the abnormal expression of mutant stem-cell clones involved in the earliest steps of skin carcinogenesis as shown by two-stage chemical carcinogenesis experiments. Rcup, a small GTPase of the Rho-superfamily, which has been shown to trigger the transition from the primary dormant to the secondary metastatic stage of carcinogenesis, is involved in the regulation of cell movement, polarity, adhesion, gene transcription, cell cycle progression and immune evasion.

Methods: One way to study basal-suprabasal communication is by using mouse models which express genes under the control of the involucrin promoter targeting specifically terminally differentiated cells. We created a mouse model expressing GFP together with Rcup Q61L, an active form of Rac under the control of the involucrin promoter targeting specifically terminally differentiated cells. We observed an increase in keratinocytes in the suprabasal layer and found that the knock of Rcup might be affected by ROS levels as Rac is part of the NADPH oxidase complex NOX2/p-glycoprotein, the most important in keratinocytes. Further experiments will show if a ROS influences with junctional formation or TGFβ responsiveness. Results: Rac overexpression by different cells lead to epidermal atrophy, hyperkeratosis, parakeratosis, hypergranulosis, synapsis and mild dermal lymphatic infiltration. At the age of 8–9 months spontaneous tumour formation was observed in about 0.2% of transgenic mice including squamous cell carcinoma (from moderate differentiated SCC to spindle cell carcinoma) and papillomas. Increase of cell colony number in site were demonstrated by colony forming assay using feeder cells. Growth was enhanced and expansion of Keratin 14 positive basal cells were noticed. Transmission electron microscopy showed the increase in number of dense core and especially in the basal-suprabasal zone, clumped keratin filaments as well as strong interaction with the basement membrane. Summerfield et al suggested that Rac might be affected by ROS levels as Rac is part of the NADPH oxidase complex NOX2/p-glycoprotein, the most important in keratinocytes. Further experiments will show if a ROS influences with junctional formation or TGFβ responsiveness.


Introduction: Melanoma is a particularly deadly cancer with a high rate of local and systemic recurrence. Recently, the potential of adoptive lymphocyte transfer and vaccination approach to enforce cellular immune surveillance has been highlighted. Several groups have demonstrated the immunotherapy efficacy with melanoma cell autologous-antigen-specific T cells. However, some tumor cells are able to survive, evade immune cell surveillance, and are able to metastasize and promote the development of dedifferentiated tumor cell subpopulations. Here, we examined whether T cells could be used to detect autotumors that would otherwise escape immune surveillance.

Results: Rac overexpression by different cells lead to epidermal atrophy, hyperkeratosis, parakeratosis, hypergranulosis, synapsis and mild dermal lymphatic infiltration. At the age of 8–9 months spontaneous tumour formation was observed in about 0.2% of transgenic mice including squamous cell carcinoma (from moderate differentiated SCC to spindle cell carcinoma) and papillomas. Increase of cell colony number in site were demonstrated by colony forming assay using feeder cells. Growth was enhanced and expansion of Keratin 14 positive basal cells were noticed. Transmission electron microscopy showed the increase in number of dense core and especially in the basal-suprabasal zone, clumped keratin filaments as well as strong interaction with the basement membrane. Summerfield et al suggested that Rac might be affected by ROS levels as Rac is part of the NADPH oxidase complex NOX2/p-glycoprotein, the most important in keratinocytes. Further experiments will show if a ROS influences with junctional formation or TGFβ responsiveness.

Discussion: The use of cyclodextrins enables a new form of loading textile materials with antiseptics that can penetrate into the fabrics. The stability of the CD-antiseptic complexes, the skin compatibility and the potential of the complexes for further applications is still an open question. Further studies are necessary to determine the best strategies for the use of textile materials with antiseptic properties.
current guidelines and try to identify an underlying cause in their cell patients. The rate of successful identification of acne was seen to be 34% on average. Since this results also showed that one out of four patients is referred to a specialized clinic or center, we analyzed the diagnostic approaches and outcome of diagnostic programs of dermatologists. Moreover, expert-report-to-interview, tertiary urtiaria referral centers were assessed for their knowledge of the current guidelines as well as their programs to identify underlying causes of acne. The most frequent reason for visiting a non-university hospital Department of Dermatology were reported. Reasons were: skin complaints, examination of skin diseases, and consultations per month on average. Ninety-five percent claimed to be familiar with the current guidelines. All centers reported to have programs for the identification of underlying causes. While most programs included only hospital visits to perform laboratory tests such as a differential blood count and determination of BSC/CRP, all other selectable diagnostic options mentioned during the interviews were chosen slightly less often (detection of thyroid hormones and anti-thyroid antibodies (38%), microbiological examination (35%), detection of total IgG (35%), consultation of an ESF-specialist (35%), uricolaxy (35%), detection of specific allergens and allergen-low diet (35%), detection (48%), consultation of a dentist (85%), skin prick testing (50%), autologous serum skin test (35%), provocation tests (78%). As underlying causes of cell, the centers reported to identify most commonly Infections (41%), drugs (20%), intolerance (17%) and auto-allergy (36%).

Conclusions: Virtually all of the participating centers attempt to identify underlying causes in cell patients by using a broad spectrum of different methods. This leads to a successful identification of underlying causes in almost half of the patients, which seems to be considerably more successful as compared to the private practice setting.
Macrophages as sentinels directing the quality of skin repair

The behavior of macrophages is determined by their oxidative state (ROS), which is closely related to their aging. The role of ROS in macrophage aging is still controversial. Here we address the question whether alterations in the redox state of macrophages might be involved in fibroblast senescence. Redox state of triple reporter fibroblasts was assessed by the measurement of intracellular ROS and GSH/GSSG ratio. A proteomic approach with 2D fluorescence difference gel electrophoresis and mass spectrometry revealed that the oxidative state of triple reporter fibroblasts was increased in senescent fibroblasts compared to young fibroblasts. In young fibroblasts, the oxidative state showed only minor changes. The redox state in fibroblasts generative lead to severe disintegration of redox homeostasis. Increased free radical production and activity were confirmed using immunostaining/blot and activity assays in vitro and in vivo, and interestingly, also in the skin of old individuals compared to young young individuals. Intracellular H2O2 concentrations were found to be increased in senescent fibroblasts following adenoviral transduction of the highly specific biotin Hunter. Using in vitro techniques like DCF staining in the presence of distinct ROS scavengers on cryosections, H2O2 was found to be increased also in skin sections from old individuals compared to young individuals. The free radical theory of ageing postulating increased concentrations of reactive oxygen species (ROS) was based on the assumption that increased ROS concentrations result in cellular dysfunction and aging. The results obtained in H2O2 knock-out mice confirmed this hypothesis. In summary, oxidative stress and ROS concentrations play an important role in fibroblast senescence. The finding that ROS concentrations are increased in senescent fibroblasts may lead to the conclusion that ROS concentrations might be involved in the aging of fibroblasts and thus in the aging of connective tissue. This is consistent with the findings that a decrease in ROS concentrations leads to an age-related improvement of skin function. In conclusion, our findings support the hypothesis that ROS concentrations are increased in senescent fibroblasts and thus in the aging of fibroblasts and thus in the aging of connective tissue.

P267

Novel findings on SERPINs as critical regulator of skin repair

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Facile and gene internal regions. Three genes (CSB, XPB, XPD) are involved in transcription elongation of RNA polymerase I, the other two are currently under investigation. As shown in Table 1, the expression of 1-ACT activity at the wound site. This finding was unexpected because up to date the liver has been shown to be the major source of 1-ACT. In addition, expression of 1-ACT mRNA was detected in skin tissues. In contrast, 1-ACT activity is predominantly found in the liver and not in the skin. These findings suggest that 1-ACT expression is regulated by different mechanisms in different tissue types. In conclusion, our study provides new insights into the regulation of 1-ACT expression and expression of 1-ACT activity in human skin disease.
P271 Increased EGR activity induces epidermal thickening and retards the initiation of hair follicle cycling in Dsk5 mice


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The epidermal growth factor receptor (EGFR) plays an important role in the homeostasis of the epidermis and hair follicle (HF). Accordingly, its de-regulated activity results in disorders as inflamma-
tory responses, tumorgenesis, and impaired wound healing. Mice completely lacking EGFR during embryonic development die few weeks after birth, depending on the genetic background. Surviving
EGFR-deficient mice and mice carrying hypomorphic mutations of the receptor develop a delayed and
disturbed hair cycling and are resistant to the growth factors of the epidermis acting as mitogens for the
hair bulbs. EGFR-deficiency does not affect the epidermal architecture, but results in an increase of HF
proliferation and an earlier onset of hair growth. Hence, the EGFR signaling pathway plays an impor-
tant role during HF development. Recently, EGFR-deficient mice were found to have increased tyrosine
kinase activity. In this study, we analyzed the long-term effect of EGFR deficiency on the thickness
of the HF bulge region in Dsk5 mice, which carry a hypomorphic mutation in the EGFR gene as well
as a null mutation in the HRAS gene. In contrast to the control littermates, Dsk5 mice showed an
altered HF morphogenesis between Dsk5 mice and control littermates at postnatal day 8.5. However, the thickness
of both the epidermis and the dermis of Dsk5 mice was significantly increased at this stage as com-
pared to control littermates. The thickness of the dermis was not affected. We suggest that the increased
expression of various nuclear retinoic acid receptors (RAR) such as 9-cis-RA, 13-cis-RA, all-trans-RA and
trans-retinoic acid (TRA) in the epidermis of Dsk5 mice was confirmed by quantitative real-time
PCR. The increased expression of these receptors is in accordance with the increased keratinizing pu-
rpose of the keratinocytes, which is supported by the increased expression of K16, which served as a marker for
keratinocytes of the hair root. Increased EGFR activity induces epidermal thickening and retards the
initiation of hair follicle cycling.

P272 Outer root sheath melanocytes, arts and grafts

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Vitiligo is a local skin depigmentation disorder, due to the lack of melanocytes in epidermis or their
function. Vitiligo occurs in 0.5% of the North European population and as high as 8% in the regions where
dark skin prevails. The symptoms are usually fully developed by the age of 20. The white patches, even though physiologically benign, bring about serious psychological disturbance and trau-
matic effects.

Conservative therapies for Vitiligo remain palliative and short-term. In contrast, therapies are being developed that target the therapy of Vitiligo and analyses about the genetic background of Vitiligo are performed. Increased EGFR activity induces epidermal thickening and retards the initiation of hair follicle cycling. 

P273 Epidermal calcium concentrations in murine atopic dermatitis visualized by Fluorescence lifetime imaging

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The disturbed skin barrier plays an important role in the pathogenesis of atopic dermatitis (AD). The disturbed skin barrier function in AD is only partly known. Besides loss of function mutations in the filaggrin gene in about 30% of the patients there are data suggesting a secondary impairment of the skin barrier by mediation of inflammation. In line with this we found decreased Si-lagrin expression in skin from patients with or without filaggrin mutations. In addition, we previously described a disturbed expression of the cornified envelope proteins involucrin and loricrin in AD. Transcripts of the gene encoding for the epidermal calcium binding protein transthyretin (TTR) were increased in skin from patients with AD. The disturbed skin barrier function in AD is associated with an increase of inflammatory cytokines, increased epidermal calcium and an influence of external calcium on eczema.

We here are using a defined inducible murine atopic dermatitis model, OVA, to assess changes of the epidermal calcium distribution dependent to normal skin by using two-photon fluorescence lifetime imaging microscopy (FLIM).

Atopic dermatitis is associated with an impaired epidermal barrier function, increased epidermal pro-

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P274 Knockdown of the novel retinal transporter STRA6 leads to hyperproliferation of keratinocytes in 3D skin equivalent models


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The STRA6 protein is a neutral, non-toxic, biodegradable polimer. It is also known as a high affinity specific membrane receptor for retinoil-RBP in bovine retinal epithelial cells. STRA6 removes retinoids from RBP and transports it across the plasma membrane into the cytoplasm. To determine whether similar transport processes take place in human skin cells, we analyzed expres-
sion of STRA6 in normal and in vitiligo skin samples. STRA6 knockdown led to increased keratinocyte proliferation and cell cycle progression, which was confirmed by immunochemical staining for keratin 16 (K16), which served as a marker for keratinocytes of the hair root. A high affinity transport system for compounds, which are able to retain active substances in the course of preparation and gradually release the retained material, is of high interest. The STRA6 knockdown leads to increased keratinocyte proliferation and cell cycle progression, which was confirmed by immunochemical staining for keratin 16 (K16), which served as a marker for keratinocytes of the hair root. A high affinity transport system for compounds, which are able to retain active substances in the course of preparation and gradually release the retained material, is of high interest.
Interleukin-1α interferes with the balance between proliferation and differentiation through insulin resistance in human keratinocytes

Vimplication for porosity pathways

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The epithelial adhesion molecule collagen XVII represents a transmembrane component of hemidesmosomes. In previous studies we have extensively analyzed shedding of human collagen XVII within its extracellular linker domain NC3A4. This is catalyzed by metalloproteinases of the ADAM family and depends rather on structural molecule motifs than on specific amino acid sequences. Here we report the generation of a recombinant domain of the human and mouse collagen XVII sheds have been directly obtained from the full collagen XVII constructs with defined deletions within their linker domain. In previous studies we have already analyzed the human collagen XVII deleted in linker domain deletion constructs of murine collagen XVII. Their normal membrane integration and Galg transition was demonstrated by cell surface biotinylation and Enol H2 in solubility. The deleted collagen of human collagen XVII resulted in non-shedding of a 20 amino acid deletion spanning Ala513 to Gln532. In contrast, deletion of 20 corresponding amino acids in murine collagen XVII (Glu494 to Glu513) do not result in reduced shedding. It revealed that the shortest deletion in murine collagen XVII which led to complete loss of shedding was a 32 amino acid deletion, from Lys513 to Ser544. Consequently, we suggest that the amino acid residues from Lys513 to Ser544 have an important role in collagen XVII shedding. The deletion of these residues is consistent with our previous findings. The different effects of the murine and human collagen XVII deletions on shedding might be explained by the different amino acid residues in the extracellular linker domain NC3A4.

In conclusion, since the extracellular linker domain of collagen XVII is largely conserved in human and murine collagen XVII and the murine collagen XVII constructs have been generated in vitro, the different effects on shedding might be explained by the different amino acid residues in the extracellular linker domain NC3A4.
focus on the impact of this directed adhesion. Surprisingly, the proliferation of BMCMCs was observed, but cell differentiation into an adipocyte dedifferentiation of adipocytes. Adipocyte differentiation is mainly controlled by a transcriptional

domain, the synthetic neolignan dihydrodehydrodiisoeugenol was assessed for its capacity to (i) increase adipocyte differentiation and to (ii) inhibit the differentiation of preadipocytes to adipocytes and (iii) maintaining the dedifferentiation of adipocytes. Adipocyte differentiation is mainly controlled by a transcriptional cascade involving peroxisome proliferator-activated receptor γ (PPAR-γ) and members of the CCAAT enhancer binding protein (CEBP) family of transcription factors.

As in silico approaches had revealed that some neoglucosides present a PPAR-γ ligand binding domain, the synthetic neoglucoside dihydrodehydrodiisoeugenol was assessed for its capacity to (i) increase adipocyte differentiation and to (ii) inhibit TNF-α and (iii) inhibit the differentiation of adipocytes under inflammatory conditions. The induction of the differentiation process was controlled for upregulation of differentiation specific transcription factors such as PPAR-γ and PPAR-β/δ, but also for induction of fatty acid binding protein 4 (FABP4) and adiponectin and for down-regulation of the preadipocyte marker PPARγ2 (PPARγ2) and a human cell culture system. For this purpose, the differentiation process of murine 3T3-L1 preadipocytes was started at confluence and studied for another 10 days using a standard differentiation regimen and a human cell culture system. For this purpose, the differentiation process of murine 3T3-L1 preadipocytes was started at confluence and studied for another 10 days using a standard differentiation regimen and a human cell culture system. For this purpose, the differentiation process of murine 3T3-L1 preadipocytes was started at confluence and studied for another 10 days using a standard differentiation regimen.
P292

Two-dimensional luminescence imaging of pH in vivo
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Abstracts

Abstract

Moreover, dihydrodihydroxydiosgenin was able to partially replace a PPARγ agonist such as tigecycline in experiments involving a two-screen approach (in vitro, dihydrodihydroxydiosgenin presented PPARγ binding capacity underlying its role as at least partial PPARγ agonist). Finally, the compound was also able to inhibit basal lipolysis in mature human adipose tissue. The two-screen approach revealed that the natural compound dihydrodihydroxydiosgenin might be well suited to over come phenotype by a dual mechanism that is increasing adipocyte differentiation and inhibition of lipolysis.

P293

Characterization of the protective immunomodulation of probiotic bacteria in local oral infections
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Abstract

Abstract

The commensal yeast C. albicans is present in about 50% of the oral cavity of healthy humans and the main-agent of fungal-cased diseases in humans. Usually, C. albicans is part of the normal mico-flora of the oral cavity and the vagina and can become pathogenic when the host immune system is weak. Several probiotic Lactobacillus species are described, that exert inhibiting and/or growth inhibiting activities on C. albicans and other infections in vivo. Therefore we choose L. rhamnosus GG to investigate the effect of this species on localised C. albicans infections and the mucosal innate immune system. Using a model system of localised candidiasis based on reconstituted human oral epithelium (RHE) we investigate a number of different aspects of host/Candida interactions as well as the effects on the RHE. Using a model system of localised candidiasis based on reconstituted human oral epithelium (RHE) the results are analyzed as raw data as well as after standardization (ST) to RH.

P294

For both ammonium ions of the skin surface and transcutaneous carbon dioxide partial pressure a significant negative correlation to skin surface pH on the FA. Furthermore, after ST to RH a highly significant correlation between ammonium and pH (r = 0.497; p < 0.001) was found on the FA. Also after ST to RH, a significant correlation (r = 0.347; p < 0.05) was found on the FH. A positive correlation between ammonium and pH (r = 0.428; p = 0.018) was found on the FH. The most prominent results from the correlation analysis were a significant negative correlation between ammonium and pH on the FH (r = -0.493, p < 0.001) and a significant correlation between ammonium and TEWL on the FH (r = 0.413, p < 0.05). The pH results for the FH were not significantly different from the TEWL results, making this finding less likely due to a dual mechanism that is increasing adipocyte differentiation and inhibition of lipolysis.

P296

The impact of beta integlin signalling on adult human hair follicle epidermal progenitor cell viability
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Abstract

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In previous studies with barium-diatrizoate as the radiopaque medium and mouse skin models, we have shown that antigens presented can be targeted using different types of particulate carriers, e.g. polyethylsuccinylated (PS) and modified vaccinia Ankara (MVA). In this study, we used biodegradable poly lactic acid (PLA) particles as well as PS particles for the transcutaneous delivery of the HIV-1 p24-peptide as well as for the targeting and activation of Langerhans cells (LCs). In vitro experiments we found that both PLA and PS particles were internalised by LCs, delivered the surface-adhered HIV-2 p4 peptide and induced the expression of maturation markers. Upon topical application on the skin surface, particles released the peptide, which was then released the adenosine. Peptide-loaded PS particles were detected in LCs isolated 16 h after particle topical application on excised human skin. Up-regulation of CD86 and CD83 along with adhesion
regulation of CD1 a surface molecules was observed after treatment with both p24-loaded PS and PLA particles. Thus, both particle types allowed for the delivery of HIV-1 p24 peptide and the modulation of skin immune system. Both mechanisms, i.e. transcutaneous delivery of particles and particle-based delivery of adsorbed antigens, may open interesting new transcutaneous vaccination strategies and skin cell targeting.

P298
IL-24 plays a key role in cutaneous wound healing via signaling through IL-22R1/IL-20R2 receptor complex
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Cutaneous wound healing is a complex regenerative and immunological process, and its disturbance represents a great medical problem. In the past, we demonstrated that the novel IL-10 cytokine family members IL-22 and IL-20 play a major role in psoriasis and skin homeostasis. Here, we studied whether these cytokines as well as another closely related cytokine of this family, IL-24, play a role in cutaneous wound healing. Interestingly, using an in vivo mouse model, IL-24 was almost constantly expressed upon wounding and following healing process, whereas IL-22 was not expressed at all. However, IL-24 was highly upregulated in the early phase of wound repair. The major sources of IL-24 appeared to be T cells and keratinocytes. IL-24 shares the IL-22R1 receptor subunit with IL-22 for mediating its biological activity. Importantly, mice lacking IL-22R1 (IL-22R1-/-) showed a delayed wound closure starting in the early phase of the healing process. Further studies identified keratinocytes, but not dermal fibroblasts, endothelial cells, melanocytes, or subcutaneous adipocytes as being targets of IL-24 action. The IL-24 treatment of human keratinocytes from both conventional cultures and three-dimensional human epidermis model regulated the expression of many differentiation-associated genes and chemokines. This study suggests that the IL-24/IL-22R1 system plays a key role in the inflammatory phase of cutaneous wound healing.

P299
The dermcidin-derived antimicrobial peptide DCD-1L forms oligomeric structures and kills bacteria by interaction with the bacterial membrane
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Dermcidin (DCD) is an antimicrobial peptide, which is constitutively expressed in eccrine sweat glands. By post secretory proteolytic processing in sweat the dermcidin protein gives rise to anionic and cationic DCD-peptides with a broad spectrum of antimicrobial activity. We could show that Dermcidin-derived peptides inhibit significantly bacterial macromolecular synthesis (RNA, DNA, protein) within the first minutes without binding to microbial DNA or RNA. Recent structural analysis indicated that the anionic 48 mer peptide DCD-1L forms ion-dependent oligomeric structures which are able to interact with the bacterial cell envelope and perturb the bacterial membrane structure. Further investigations by CD-spectroscopy and conductance measurements with artificial phospholipid membranes suggest that DCD-1L is able to form small pores in the bacterial membrane which leads to ion efflux and bacterial death. These data show for the first time how an antimicrobial peptide present in human eccrine sweat is able to kill efficiently several types of microorganisms.
TGF-beta P074, P150 (V01)
Th1/Th2 P002 (V22), P003, P018, P145 (V24), P180, P184, P216, P275
TNF-alpha P092, P146, P155, P169, P279 (V29)
Transcription P269
Transcription factors P029, P043, P116, P143, P207, P244 (V28), P249
Transfection P022, P026, P027, P132, P133, P136, P139
Transgenic mice P253 (V02)
Transglutaminase P275
Tumor progression P040 (V11), P072, P080, P111, P145 (V24), P152, P187, P200 (V33), P209 (V05), P213, P214, P215, P216, P222, P226, P227, P233, P247
Tumor promoter P252 (V17), P255 (V25)
Tumor suppressor gene P217, P238 (V35)
Tyrosinase P221 (V15)
Tyrosine kinase P199
U
Ultraviolet P046, P060, P193, P194, P195, P196, P197, P198, P283, P288
V
Vaccine P128, P129, P134, P136, P138, P148, P161, P165, P166, P253 (V02)
Vasculitis P188, P207
VCAM-1 P284
VEGF P073, P213, P214
Virus P070, P108, P140, P188, P212
Vitiligo P272
W
Warts P189
Wound healing P026, P055, P074, P172, P256, P268, P263, P267, P288, P292, P298