The anticycmetics suppress the allergic symptoms of psoriasis in the skin of psoriasis patients. The treatment led to a significant reduction in the severity of psoriasis. The skin lesions were observed after the treatment. The levels of serum IgE, tissue IL-4, IFN-γ and IL-17A were measured with quantitative real-time polymerase chain reaction (PCR). Results. Similar to the analysis of IgE, QP could significantly inhibit the induced AD lesions in mice. The treatment led to a significant reduction in the severity of AD. The levels of serum IgE, tissue IL-4, IFN-γ and IL-17A were measured with quantitative real-time polymerase chain reaction (PCR). Results. Similar to the analysis of IgE, QP could significantly inhibit the induced AD lesions in mice. The treatment led to a significant reduction in the severity of AD. The levels of serum IgE, tissue IL-4, IFN-γ and IL-17A were measured with quantitative real-time polymerase chain reaction (PCR).
Low numbers of memory T cells correlate with minor CCL27 expression in prenatal human skin

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CCL27, a chemokine, is expressed in the epidermis of adults, is a key chemokine attractant for CLA+ memory T cells. Recently, it has been reported that, in contrast to adult skin, prenatal skin harbors only few memory T cells. In this study, we investigated whether this scarcity correlates with CCL27 levels during gestation in vivo and analyzed its expression in fetal skin. The cytokine was not detected in fetal skin and in adult skin, as previously reported. Our data showed that CCL27 is a key chemokine attractant for CLA+ memory T cells seen in adult skin. However, the scarcity of CLA+ memory T cells in human prenatal skin is not due to a lack of CCL27 expression in the fetal skin.

The early environment in the womb, including the immune system, is crucial for the development of the immune system. CLA+ memory T cells were found in adult skin but not in fetal skin. However, in prenatal human skin, CCL27 was present in supernatants of ex-vivo skin cultures derived from adult skin samples but was absent in supernatants of prenatal skin. Similarly, CCL27 was produced and secreted in vitro by adult skin but not by fetal skin. Therefore, CCL27 expression in the skin is not only regulated by cytokines but also by the developmental stage of the skin. The scarcity of CLA+ memory T cells in prenatal skin is partly due to the lack of CCL27 expression in the skin.

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TNF-α and IL-17A are cytokines that are involved in the development of psoriasis. Therefore, we investigated whether TNF-α and IL-17A are involved in the development of psoriasis in prenatal human skin. Our results showed that the expression of TNF-α and IL-17A is significantly lower in prenatal skin as compared to adult skin. These findings suggest that the development of psoriasis in adults is not only caused by the presence of cytokines but also by the developmental stage of the skin. The scarcity of CLA+ memory T cells in prenatal skin is partly due to the lack of CCL27 expression in the skin.

In conclusion, the expression of CCL27 in the skin is not only regulated by cytokines but also by the developmental stage of the skin. The scarcity of CLA+ memory T cells in prenatal skin is partly due to the lack of CCL27 expression in the skin. Therefore, the development of psoriasis in adults is not only caused by the presence of cytokines but also by the developmental stage of the skin.

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Expression of IL-23/Th17 pathway in early inflamed acne lesions

Inflammation of the skin is orchestrated by various immune responses. The role of the IL-23/Th17 pathway in the development of acne vulgaris is not fully understood. Here we aimed to characterize the IL-23/Th17 pathway in early inflamed acne lesions.

We employed immunohistochemical staining using IL-23 and IL-17A as markers for the IL-23/Th17 pathway. The results showed a significant increase in the expression of IL-23 and IL-17A in early inflamed acne lesions compared to non-inflammatory skin. These findings suggest a crucial role of the IL-23/Th17 pathway in the development and progression of acne vulgaris.

Conclusion: The IL-23/Th17 pathway plays a significant role in the development of early inflamed acne lesions. Further studies are needed to elucidate the mechanisms underlying this pathway in acne vulgaris.

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ABSTRACTS | Adaptive Immunity

019

Interleukin- and tumour necrosis factor control human cancer by induction of cellular senescence

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Recent studies from targeted cancer immunotherapies show that adaptive immunity can control human cancer. Cancer control occasionally occurs in the absence of cytotoxic effects, e.g. induction of apoptosis or perforin/granzyme B-mediated cytosotoxicity. Interestingly, we and others found that tumour-specific, interferon-γ and tumour necrosis factor (TNF)-producing T helper (Th1) cells arrest cancer growth, but the underlying mechanisms were unknown. As MHC class II-restricted Th1 cells can not directly interact with most cancer cells, we investigated the effect of soluble Th1 cytokines on human cancer cells. Proliferation was measured by BrdU incorporation, cell cycle regulation by Western blot analysis of the p38/42 MAPK signalling pathway, and senescence induction by an in vitro growth arrest assay. Treatment of A2780 rhabdomyosarcoma cells with IFN-γ and TNF for 24 h strongly suppressed their proliferation. Growth inhibition was accompanied by severe hypophosphorylation of Rb at Ser795 indicating that the combined action of the two cytokines drives cancer cells into senescence. Indeed, the cell cycle arrest was permanent, as the cancer cells did not recover after 48 h of cytokine deprivation. 55% of the cell population exhibited senescence-related CDK arrest with induction of senescence in primary human rhabdomyosarcoma and melanoma cells. Taken together, our data show that Th1 cytokines control human cancer by inducing irreversible growth arrest. Thus, cytokine-induced senescence can be considered as an integral part of tumor surveillance, and opens perspectives for innovative immunotherapeutic approaches.

020

The amino acid sequence EDExx in the intracellular domain of the DEC-205 receptor mediates antigen uptake in dendritic cells by interaction with adaptin-2

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The antigen receptor DEC-205 mediates efficient endocytosis in dendritic cells (DC). Its intracellular domain harbours a putative targeting sequence (single letter code: EDExx), which is highly homologous to a DiExx sequence previously shown to route lipoprotein receptors to lysosomes. To analyze the function of this motive in DEC-205, we generated fusion receptors containing the tB-globulin binding extracellular domain of human CD16 and the murine intracellular DEC-205 domain (WT-DEC/CD16). By site directed mutagenesis we also mutated the EDExx sequence to AAAxx (AAA-DEC/CD16). Stably transfected cell lines of DCEK cells were established. When we analyze the endocytosis of the ligand hIgG(tB-globulin) in the two different cell lines we found that WT-DEC/CD16 cells took up the ligand completely within 30 min, whereas AAA-DEC/CD16 endocytosed only 10% of the hIgG(tB-globulin). When analyzing the distribution of the DEC/CD16 receptors in steady state, i.e. without incubation with hIgG, we found that WT-DEC/CD16 receptors were present in vesicles within the cells, whereas AAA-DEC/CD16 endocytosed receptors remained mostly close to the cell surface. Analysis of the trafficking of IgG(tB-globulin) showed that WT-DEC/CD16 receptor recycling to the cell surface was faster than in AAA-DEC/CD16 cells. Moreover, the recycling of the WT-DEC/CD16 receptor was blocked by the addition of brefeldin A (BFA). These data imply that the AAAXX motive in the intracellular domain of the antigen receptor DEC-205 is involved in the interaction with adaptin-2 and with the EDEXX motive in its intracellular domain. This interaction is crucial for the efficient presentation of antigens by DC and may therefore influence the effective activation of T cells by MHC-peptide complexes.

021

Pathodynamics of DBM cell trafficking in a murine model of graft-versus-host disease (GVHD), implicate the CXCR3 axis as a crucial organizer of cutaneous inflammation and injury

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Thymic emigration is the most commonly affected organ in GVHD, the mechanisms underlying its development are incompletely characterized. To study early molecular processes, qRT-PCR was used to profile epidermal chemokine mRNA expression in a model of GVHD-like disease where K14-cre mice co-inoculated with 5% SV40+ and 95% SV40- bone marrow chimeras (CMR) develop skin inflammation and weight loss following adoptive transfer of OT-I (KOA-specific) CD8+ T cells. qRT-PCR of epidermal mRNA was done 24hr post-propagation of C57BL/6 (KOA+CXCL9/CXCL10, a 1% lidocaine-elicited skin injury model) and a common CCR3 receptor, with 10 fold induction of CXCL10 mRNA detectable as early as 2 days post-OT-I transfer (dpi) and peak induction of CXCL9 and CXCL10 mRNA at 100 and 150-fold, respectively, at 5 dpi. At 2 to 4 dpi, qRT-PCR of skin-draining lymph nodes (SDN) revealed early upregulation of CXCL9 and CXCL10 mRNA from between 20-40 fold vs. untreated K14-cre+DBM CMR mice depicting that CXCL9/10 mRNA expression in the SDN is a major inducer of immune infiltration into skin. Coincident with peak epidermal CCL10 mRNA expression, CD8+ T cells were detected in the skin as early as 5 dpi via IF and progression of skin injury, persisting beyond 13 dpi. SDN FACS of total skin suspensions showed that CD8+ T cells accounted for 31.8% of total skin lymphocytes. The kinetics of chemokine and receptor expression suggested that CXCL9/10 may accelerate migration of Ag-specific T cells to antigen presenting target organs. Transfer of CH50XCMO cells into K14-cre+DBM mice resulted in unaltered weight loss, but markedly attenuated skin disease vs. wild-type OT-I transferred K14-cre+DBM controls. These findings indicate that, in GVHD, epidermis expresses CXCL9/10 and CCR3+CD8+ T cells and suggest that targeting the CXCR3 axis may have clinical utility in GVHD and related skin diseases.

022

GILT expression in medullary thymic epithelial cells is required for presentation of tissue-specific self antigens and deletion of autoreactive T cells

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Clonal deletion of thymocytes with a high avidity for self-antigen-MHC complexes is a crucial step in the maintenance of tolerance and prevention of autoreactivity. We have previously shown that gamma-interleukin-inducible lysosomal thiol reductase (GILT) is required for thymic deletion of CD4+ T cells specific for the self and melanoma antigen, tyrosinase-related protein 1 (TRP1). To define GILT’s role in the maintenance of central tolerance, we showed that GILT expression is enriched in thymic antigen presenting cells (APCs) capable of mediating deletion, namely medullary thymic epithelial cells (TECs). In contrast, a large number of TRP1-specific T cells are deleted in thymi in which GILT is expressed in both TECs and bone marrow-derived APCs in which GILT expression is restricted to TECs, demonstrating that GILT expression in TECs is sufficient for deletion of TRP1-specific thymocytes. Here, we analyzed the requirement for GILT expression in BM-derived APCs in medullary thymic tissue. In chimeric CD16+ TEC BM chimeras, GILT expression was deleted specifically from BM-derived APCs in the thymus. Thymocytes from these mice were unresponsive to the deletion of TRP1-specific T cells, confirming that GILT expression is required for deletion of TRP1-specific thymocytes. These findings suggest that GILT operates in TECs to facilitate the presentation of tissue-specific self antigens and promote deletion of autoreactive T cells.

023

Characterization of house dust mite specific T cells in atopic dermatitis at the single cell level

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House dust mite (HDM) is one of the most important allergens in atopic disease (AtD). Despite the advances in the pathogenesis of AtD, the clinical relevance of HDM-specific T cells remains unclear. Here we investigated HDM-specific T cells at the single cell level. CD4+ T cells (Tetramer+/CD45R0+) in the peripheral blood of sensitized patients with AD. We confirm observations from asthma research and describe HDM-specific T cells have been reported recently and are described as central and effector memory T cells producing IFNγ, IL-4 and Th17 cytokines in patients with atopic dermatitis (AD). By subsequent fluorescence-staining/bleaching steps, immobilized cells are analyzed by a comprehensive marker set, providing information about the T cell subset. Furthermore, the staining is documented in a microscopy picture, allowing about the T cell subset. Furthermore, the staining is documented in a microscopy picture, allowing

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025
Anaphylaxis triggered by innate immune signals as co-factors is mediated by basophils independent of IgE
F. Welte, S. Kaseler, W. Kempf, B. Sirakou, P. Eckstein, M. Krohne, V. Skalicky, F. Lang, P. H. D. Welte, M. Röcken and B. Budde1, 2 Department of Dermatology, Eberhard Karls University, Tübingen, Germany, 3 Institute of Immunology, Philipps-University, Marburg, Germany, and 4 Department of Infection Biology, Friedrich-Alexander University, Erlangen, Germany.

Anaphylaxis is classically induced by cross-linking of IgE bound to FceRI on mast cell, MC, or basophil surfaces. Alternatively, IgG antibodies can also initiate anaphylaxis. Certain co- or augmentation factors, as best documented for wheat dependent exercise induced anaphylaxis, alcohol consumption or infections, can augment anaphylaxis. To understand how infections trigger anaphylaxis we sensitized mice with Ovalbumin-OVA and mimicked infection by pretreatment with different pathogens as well as different molecular patterns (PAMPs) prior to challenge. Pretreatment triggers full-blown anaphylaxis as measured by significantly decreased core body temperature, significant reduction in systemic blood pressure and an increase in serum histamine levels. Experiments using basophil depleted or IgE deficient lacZ transgenic mice showed that PAMP triggered anaphylaxis is MC independent but basophil dependent. Most interestingly, IgG knock-out mice in which the eosin for IgG1 is substituted by an eosin coding for IgG1 leading to IgG1 deficiency and IgG over production did not develop basophil dependent anaphylaxis. Finally, to quantify the amount of PAMPs acting on basophils triggering IgG1 dependent activation, we generated knock-out mice deficient in the pathogen recognition receptors TLR2 and TLR4, and we measured the analysis of the PAMP PGN. In conclusion, our results show that PAMPs act as co-factors modulating the onset of IgG1 and basophil dependent anaphylaxis via binding to TLRs. These data show for the first time a clinical relevance of IgG1 basophil independent mediated anaphylaxis. This is of major clinical importance for the diagnosis and management of patients with anaphylaxis and may help to develop to new therapeutic strategies.

026
Nanoparticle based intradermal formulation targeting DC skin subsets to increase vaccine potency against HIV-1
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Delivery of vaccine formulations into the dermis using antigen-coated micro needles is a promising approach due to the efficient antigen delivery and safety. There is evidence that intradermal delivery of vaccines is more effective than alternative routes such as intramuscular. Vaccines preventing HIV infection are still lacking, effective protection against this pathogen would represent a major advance in global health. The aim of this study is to evaluate the capacity of a new intradermal vaccine formulation using ovalbumin specific antigens conjugated to polyethylen glycol nanospheres (PPS-NPs) to induce immunity against HIV infection. We tested the efficacy of PPS-NP formulations on primary Dendritic Cells (DC) including skin DC subsets. Firstly we used Monoocyte-derived DCs (MoDC) and PPS-NP to formulate proteins in the presence and absence of TLR agonists before analysis for cell viability, maturation and cytokine production. From these preliminary results we can conclude that the PPS-NP formulation is non-toxic to the DC and does not affect DC maturation or TNF-α production. DCs produced TNF-α in response to LPS. DCs from different human skin samples were incubated with the PPS-NP and PPS-NP conjugates in the presence and absence of TLR agonists before analysis for cell viability, maturation and cytokine production. These preliminary results allow us to conclude that the PPS-NP formulation is non-toxic to the DC and does not affect DC maturation or TNF-α production. DCs from different human skin samples were incubated with the PPS-NP and PPS-NP conjugates in the presence and absence of TLR agonists before analysis for cell viability, maturation and cytokine production. From these preliminary results we can conclude that the PPS-NP formulation is non-toxic to the DC and does not affect DC maturation or TNF-α production. 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Abstracts

031 The expressions of T cell immunoglobulin and mucin domain molecules on peripheral blood mononuclear cells and skin lesions of sarcoidosis patients

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Sarcoidosis is a T helper (Th1) mediated inflammatory disease with unknown aetiology, characterized by the formation of noncaseating granulomas, and involving accumulations of monocyte/macrophage-lineage cells and T cells. T cell immunoglobulin and mucin domain (TIM) proteins regulate activation and function of CD4+ T cells and are differentially expressed on Th1 and Th2 cells. In humans, TIM-1 protein is associated with Th2 cell responses and TIM-3 preferentially is expressed on fully differentiated Th1 cells but not on Th2 cells. Furthermore, TIM-3 is a regulator for key pro-and anti-inflammatory cytokine production in human monocytes. In this study, we investigated whether TIM molecules could play a role in the pathogenesis of sarcoidosis. TIM-1+ cells and TIM-3+ cells in peripheral blood were significantly increased in sarcoidosis patients compared with healthy controls. The relative frequency of both T cells was correlated with the serum ACE and soluble IL-2 receptor levels. On the other hand, the relative frequency of TIM-3+ mononuclear cells and skin lesions of sarcoidosis patients were present in and around the granulomas of sarcoidal skin lesions, respectively. These findings indicate the critical role of TIM molecules in the regulation of Th1/Th2 polarization in sarcoidosis.

033 Opposing immune reactions drive psoriasis and eczema

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Recent data suggest a complex interplay of Th1 and Th2 cytokines in atopic dermatitis (AD) and psoriasis. Therefore, in the current study, we analyzed the gene expression profile of paired skin biopsies of psoriasis and eczema patients. Methods: Expression profiling of paired skin biopsies (n = 10) from 5 psoriasis and 5 eczema patients was performed using a Human Genome U133 Plus 2.0 GeneChip. Results: The analysis revealed a global down-regulation of Th1 cytokines and up-regulation of Th2 cytokines in psoriasis compared to eczema. Furthermore, Th2 cytokines IL-5, IL-13, and IL-4 were significantly up-regulated in psoriasis, whereas Th1 cytokines IFN-γ and TNF-α were significantly down-regulated. The up-regulation of IL-5 and IL-13 suggests a role for eosinophils and basophils. In conclusion, the results of our microarray analysis indicate the presence of opposing immune reactions in psoriasis and eczema. Both sets of cytokines are expressed at a mutually exclusive manner.

034 Anti-inflammatory effect of cholecystokinin in skin disorders

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Cholecystokinin (CCK) is a gastric peptide hormone, which induces the release of bile and other digestive enzymes to facilitate digestion of fat and protein. It is also known to possess immunomodulatory actions, and in recent years, CCK has been suggested to be involved in the pathogenesis of a variety of inflammatory diseases. In the current study, we investigated the potential anti-inflammatory effect of CCK in skin disorders using a mouse model. CCK was administered by the intraperitoneal injection, and the effect on the skin inflammation was evaluated. Our results showed that CCK administration significantly reduced the skin inflammation, indicating the anti-inflammatory effect of CCK in skin disorders.

035 TACE/ADAM17 deficiency in epidermis leads to IL-17A-associated inflammation with atopic dermatitis-like phenotype

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TACE (tumor necrosis factor-α converting enzyme), or ADAM17 (a disintegrin and metalloproteinase) is a membrane-bound proteolytic enzyme that regulates cell proliferation and differentiation. We recently developed TACE-/- or TACE ΔχSV mice, in which TACE/ADAM17 is absent in epidermis (TACE cKO). In this study, we examined the effect of TACE deficiency on skin inflammation. Results: TACE deficiency in epidermis resulted in a Th17-mediated skin inflammation. TACE cKO mice exhibited a characteristic Th17 skewing in the skin, characterized by increased IL-17A expression and infiltration of Th17 cells. In conclusion, TACE deficiency in epidermis leads to a Th17-mediated skin inflammation, providing a potential therapeutic target for atopic dermatitis.

036 Inhibition of STAT6 signals exacerbates IgE-mediated, basophil-dependent prurigo-like reactions

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Prurigo is a common skin disease characterized by urticarial papules and/or nodules with severe itching. This condition occurs in association with various underlying diseases, such as chronic renal failure and internal malignancies. Atopic dermatitis is also occasionally complicated by prurigo lesions. The pathological mechanisms causing prurigo remain unclear, although Th2-type immunity has been implicated in its etiology and our recent study showed a number of basophil in prurigo lesions. The present study demonstrated that repeated intradermal injection of TNP-OVA to dorsal skin of IgE-transgenic mice resulted in the development of persistent, basophil-dependent, nodular skin lesions. Histopathologically, marked hyperkeratosis with irregular acanthosis and marked expression of intercellular adhesion molecule-1 (ICAM-1) was observed using confocal microscopy. A significant expression was prominently depressed by the addition of CCK8S, particularly in the cell surface. Furthermore, RT-PCR analysis confirmed that the expression of mRNAs for ICAM-1 was also depressed by the addition of CCK8S. The present study indicates that this novel peptide is capable of exerting strong anti-inflammatory signal in the skin, and the association with inflammatory skin disorders such as AD is addressed.

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037 Regulation of Claudin-1 expression in Langerhans cells by EpCAM

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Strong antigenic stimuli in skin leads to the generation of effector memory T cells (TEM) that distribute to primary sites of inflammation, but also to the whole body surface as resident memory T cells (TRM) and confer long term protection. In the present study, we generated mice lacking only dermal γδ T cells and demonstrated that contact hypersensitivity (CHS) response to DNFB was significantly reduced by dermal γδ T cells. However, the role of skin γδ T cells in cutaneous acquired immune responses remains unclarified. In the present study, we generated mice lacking only dermal γδ T cells using bone marrow transplantation chimera and demonstrated that contact hypersensitivity (CHS) response to DNFB was significantly reduced by dermal γδ T-cell depletion. In vitro restimulation of sensitized lymphocytes revealed that dermal γδ T cells promote the sensitization phase of CHS. In addition, by means of photo-stimulating system using photo-converter protein-Kae4-transgenic mice, we demonstrated that a fraction of γδ T cells migrated from the skin to draining lymph nodes (DLNs) prominently during the sensitization phase. Majority of skin-derived γδ T cells in DLNs expressed CCR6 and Vγ4 chain, suggesting that they were dermal origin. In contrast to other skin immune cells, the migration of γδ T cells to the DLNs was independent of Gαi-coupled receptors, since pertussis toxin treatment did not prevent their migration. Moreover, coculture of dendritic cells with Vγ4-positive γδ T cells led to a significant upregulation of MHC class II molecules and IL-12 production in a TNF-α-dependent manner, which suggests that migrating γδ T cells activate the dendritic cell functions in the DLNs. Taken together, we newly identified Vγ4-positive dermal γδ T cells, which migrate to the DLNs and establish the sensitization phase of CHS by enhancing dendritic cell functions.

038 Mechanistic correlations between two itch biomarkers, cytokine interleukin-31 and neuropeptide β-endorphin, via STAT3/calcium axis in atopic dermatitis

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Itch is the cardinal symptom of atopic dermatitis (AD). β-Endorphin, a neuropeptide, is increased in both AD skin and sera. Interleukin (IL)-31, an itch-relevant cytokine, activates IL-31 receptors in keratinocytes. However, how IL-31 and β-endorphin interact in AD skin remains elusive. This study aims to investigate the mechanistic interaction of IL-31 and β-endorphin in AD. This was a prospective cross-sectional study. We recruited adult patients with AD and controls following Hanifi’s AD criteria. Serum levels of IL-31 and β-endorphin were measured by enzyme-linked immunosorbent assay. AD patients had significantly higher serum levels of IL-31 and β-endorphin compared to controls. Serum cytokine IL-31 and neuropeptide β-endorphin were significantly correlated, in patients with AD and in controls. We also treated primary keratinocytes with IL-31 and measured calcium influx, β-endorphin production and signalling pathways to define their mechanistic interactions. Our result showed that β-endorphin induced calcium influx and STAT3 activation, pretreatment with STAT3 inhibitor stopped the increase of β-endorphin. Notably, either replacement of extracellular calcium or treatment with 2-aminoethyl diphenyl borate, an inhibitor for the store-operated channel, blocked STAT3 activation. We found higher levels of blood β-endorphin and IL-31, which were significantly correlated, in patients with AD. Moreover, IL-31R and β-endorphin were increased and colocalized both in AD human skin and TCP-painted mouse skin. We concluded that IL-31 receptor activation in keratinocytes induces calcium influx and STAT3-dependent production of β-endorphin. These results might contribute to an understanding of the regulatory mechanisms underlying peripheral itch.
with Candida albicans

Langerhans cell derived IL-6 is required for the differentiation of Th17 during skin infection

DA system for differentiating human monocytes into antigen presenting cells

A system for differentiating human monocytes into antigen presenting cells

One of the major antimicrobial peptides (AMPs) secreted by keratinocytes is RNase7, a member of a range of antigen presenting cells (APCs). Experiments evaluated morphology using light microscopy, phenotype using ten parameter flow cytometry, and function using APCs co-cultured with autologous naive CD8+ T-cells in the skin and promoted Th2 differentiation in the draining lymph nodes. Consistently, the serum IgE level in IL-17^{-/-} mice was significantly lower than that in wild-type mice after repeated hapten application. We also found that the main producer of IL-17A in the lesional skin was dermal γ± CCR8+ γΦ cells, which migrated to the epidermis. To further give in-depth consideration to the above findings, we used flaky tail (ftt) mice that exhibit AD-like skin lesions with elevated IgE. We crossed Ftt mice with IL-17A^{-/-} mice and generated Ftt mouse deficient in IL-17A (IL-17A^{-/-}/ftt) mice. IL-17A^{-/-}/ftt mice showed impaired serum IgE level compared to Ftt mice in the steady state. In addition, the number of IL-4 producing cells in the skin draining lymph nodes was significantly decreased in IL-17A^{-/-}/ftt mice compared to that in Ftt mice. Moreover, epidermal thickness tended to be decreased in IL-17A^{-/-}/ftt mice compared to Ftt mice. In line with the result of the hapten-induced AD model, IL-17A^{-/-}/ftt mice in the skin of Ftt mice was γΦ γ cells. Together, our results suggest that dermal γ± γ Φ cells migrating into the epidermis are the essential sources of IL-17A in the AD-like skin lesions, and that IL-17A promotes ftt inducibility in the skin. γΦ γ cells with NOD2 activity might be directly exposed to RNase7. Here we investigated the influence of RNase7 on activated human CD4+ T-cells. In the current study we demonstrate that treatment of activated human CD4+ T-cells with RNase7 significantly reduced the production of the TH2 cytokines IL-4 and IL-13. We next aimed for an extensive side by side study on activated CD4+ T-cells in which we cannot respond to enhanced apoptosis. To test the importance of individual LC-derived cytokines, we generated a series of mice in which we abrogated expression of particular cytokines in LC alone. In contrast to IL-23 and IL-6, we failed to find a relevant regulator of the TH2 cytokine response. Furthermore, we recently demonstrated using a Candida albicans skin infection model that these DC subsets all acquire antigen but are necessary and sufficient for the differentiation of naive T cells into the Th17 phenotype while CD103+ DC require for the development of Th1. In other systems, Th17 differentiation requires the presence of TGFβ and either IL-6 or IL-17 depending on the tissue as well as IL-23 for the maintenance of the Th17 phenotype. Activated LC and dDC produce high levels of IL-12p70, IL-23 and IL-10, a pattern of cytokines which LC cannot respond to. In contrast to IL-23 and IL-6, we found that RNase7 has immunomodulatory functions on TH2-cells and might foster a TH1 cytokine environment in the skin.

A system for differentiating human monocytes into antigen presenting cells

One of the major antimicrobial peptides (AMPs) secreted by keratinocytes is RNase7, a member of the family of RNase7. RNase7 is constitutively expressed in the epidermis of healthy human skin and has been found to be upregulated in chronic inflammatory skin diseases such as atopic dermatitis and psoriasis. In addition to their antimicrobial activity, immunoregulatory functions have been published. Differentiation of murine LC into inflammatory DCs (TIP-DC) in the draining lymph nodes of patients with psoriasis and psoriatic arthritis resulted in cytokines which can be directly exposed to RNase7. Here we investigated the influence of RNase7 on activated T-cells. In the current study we demonstrate that treatment of activated human CD4+ T-cells and Th2-cells with RNase7 significantly reduced the production of the Th2 cytokines IL-4 and IL-21 by activated human T-cells while IFNγ and IL-2 were not regulated. This effect was not due to enhanced apoptosis. To analyze if RNase7 has a member of the RNase7 family, RNase7 is constitutively expressed in the epidermis of healthy human skin and has been found to be upregulated in chronic inflammatory skin diseases such as atopic dermatitis and psoriasis. In addition to their antimicrobial activity, immunoregulatory functions have been published in the literature. In particular, LC induced the expression of RNase7 in murine CD4+ T-cells. However, the role of RNase7 in human LC is not well understood. Therefore, we tested the effects of antigen presentation isolated to LC or CD103+ DC in the steady-state, we performed a series of experiments with either 1) transgenic mice that express human Langerin only in LC or 2) mice lacking LC were immunized with anti-human Langerin/antigen complexes or 2) mice lacking LC were immunized with anti-mouse Langerin/antigen complexes. In both cases the DC remained unactivated and CD103+ DC were not activated. Both DC subsets, were able to promote proliferation of MHC-II tetramer binding endogenous CD4 T cells that peaked at day 7 and slowly declined over time without evidence of deletion. LC but not CD103+ DC induced CD4 T cells to produce high levels of IL-10. In contrast, LC but not CD103+ DC induced the production of IFNγ and IL-12 in CD4 T cells. Our results show that RNase7 has immunomodulatory functions on TH2-cells and might foster a TH1 cytokine environment in the skin.

A study on the heterogeneity of CD16+ myeloid cells in human blood that serve as precursors of immunoregulatory dendritic cells

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A study on the heterogeneity of CD16+ myeloid cells in human blood that serve as precursors of immunoregulatory dendritic cells

A new role of IL-17A as an inducer for Th2 in murine atopic dermatitis

RNase7 regulates TH2 cytokine production by activated human T-cells

Langerhans cells derived IL-4 is required for the differentiation of Th17 during skin infection with Candida albicans

Langerhans cell derived IL-6 is required for the differentiation of Th17 during skin infection

RNase7 regulates TH2 cytokine production by activated human T-cells
**049**

Human th6 cells: A novel skin homing T cell subset with potent autocrine and paracrine proinflammatory properties

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IL-9 producing th6 cells have been as a novel subset of helper T cells and animal studies suggest a role in tissue immunity and in allergic/autoimmune inflammation. Prior studies have been limited to genetically modified mice or to th9 cells differentiated in vitro using Tgf-beta. Studies of memory th9 cells from human blood and tissues are conspicuously lacking. We isolated tissue resident T cells from healthy human skin, intestine and lung and found th7 cells only in skin. IL-9 was also expressed in skin lesions of psoriatic and atopic dermatitis. We next sorted blood th6 cells into 3 populations (skin tropic (CLA+), gut tropic (CD45RA-) or neither) and studied cytokine tissue production and pathogen specificity. IL-9 was produced only by skin tropic T cells and many were specific for C. albicans. IL-9 production was transient after activation, was not dependent on Tgf-beta, and th9 cells downregulated other cytokines. Expression of granzyme B and perforin was up-regulated in IL-9+ th6 cells, establishing their identity as a distinct th6 T cell subset. IL-9 receptor was highly expressed by CLA+ T cells suggesting th6 cells may be both a source and target of IL-9. Blocking IL-9 at the initiation of disease in vivo, University of Bern, Bozen, Germany, Italy and 2 Institute for Pharmacological Biology, University of Bern, Bozen, Germany

**050**

IKKβ and NF-κB may work cooperatively in CD8+ T cell effector differentiation

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We previously reported that interferon regulatory factor 8 (IRF8), a transcription factor that belongs to the IRF family, regulates TCR-mediated signaling and cytokine signaling pathways and drives effector differentiation of CD8 T cells. We demonstrated that these two signaling pathways synergistically promote the generation of cytotoxic T cells and IRF8 is persistently activated by these two signals. Blocking these signaling pathways by either ZAP70 or 5α-reductase inhibition in vitro altered IRF8 upregulation and inhibited effector differentiation of CD8 T cells. Using K14-MOVA Tg mice that develop graft-vs-host disease (GvHD) after transfer of syngeneic CD8+ T cells, we showed that CD185R8KO cells induced less severe GvHD and attenuated effector functions, including cytotoxicity and IFN-γ production. However, genetic deletion of IRF8 did not completely abrogate the GvHD response or IFN-γ expression in vivo, suggesting that other transcription factors may be involved. Since IRF8 exerts its function(s) by forming a dimer with other IRF family members, we assessed other IRF family members for pairing. Only IRF4 showed an expression pattern similar to IRF8. IRF4 was upregulated upon activation of CD8+ T cells and was suppressed by blocking TCR-mediated or cytokine signaling pathways, suggesting that IRF4 may drive effector differentiation of CD8+ T cells together with IRF8. To determine how IRF4 and IRF8 expression relates to the development of atopic dermatitis (AD), we performed real-time PCR using activated CD8+ T cells cultured in the presence of a ZAP70 inhibitor or a Jak3 inhibitor. The results demonstrated that ZAP70 and IRF4 did not show parallel expression patterns. Although IRF8 and IRF4 suggest that pairing of IRF8 and IRF4 is important, further studies are required to modulate these types of immune responses.

**051**

HIV infection predisposes skin to toxic epidermal necrolysis via depletion of skin-directed CD4+ T cells

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HIV infection with the human immunodeficiency virus (HIV) is a well-known risk factor for the development of adverse cutaneous drug eruptions (ACDEs), however little is known about the pathogenesis of ACDEs or the mechanisms by which HIV infection predisposes individuals to a higher incidence of ACDEs. Additionally, the mortality of severe ACDEs remains high, and there is need for investigation into new treatment modalities and immunological mediators that may serve as potential targets for such new therapies. To assess whether an altered immunological state contributes to the development of ACDEs in HIV-infected individuals, skin biopsies of toxic epidermal necrolysis (TEN) were obtained from acutely ill HIV-infected patients (one with AIDs and one without AIDs) and compared to healthy skin. The skin samples were characterized for the associated inflammatory infiltrates. An 8-fold increase (p=0.006) in the ratio of CD4+ to CD8+ T cells was observed in the dermis of HIV-positive patients afflicted with TEN (p=0.033) compared to healthy skin. In addition, a decrease in the total number of CD8+ T cells was observed in the dermis of HIV-positive individuals compared to healthy skin (p=0.062). Our findings suggest that the increased risk of ACDEs in HIV-positive individuals may be attributed to a shift in the CD8+/CD4+ ratio towards the effector CD8+ T cell population. The tropism of the HIV virus for CD4+ T cells may explain the substantially decreased CD8+ T cell numbers in the skin of HIV-infected patients afflicted with TEN. Furthermore, the CD8+ T cell lymphokine profile was characterized for cytokines (IL-2, IFN-γ, TNF-α and IL-17) associated with each stage of AD progression. In conclusion, we showed a heterogeneous population of CD4+ and CD8+ T cells present in healthy human skin. Cell suspensions prepared from epidermis, dermis and peripheral blood were analysed by flow cytometry for CD4+ and CD8+ T cells. The results demonstrated that ZAP70 and IRF4 did not show parallel expression patterns. Although IRF8 and IRF4 suggest that pairing of IRF8 and IRF4 is important, further studies are required to modulate these types of immune responses.

**052**

Characterisation of epidermal and dermal T cells in healthy human skin

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Healthy human skin contains a large number of T cells. Depending on the local micronvironment, skin derived T cells have the ability to act either as regulatory or effector T cells. T cell resident memory T cells (TREMs) are retained in murine skin after infection and are identified by CD103+ and CD49a expression. TREMs provide a first line of defence against secondary infection or viral reactivation. Similar T cells have been shown in human skin after HIV infection. In this study, we analysed the different subpopulations of CD4+ and CD8+ T cells present in healthy human skin. Cell suspensions prepared from epidermis, dermis and peripheral blood were analysed by flow cytometry for CD4+ and CD8+ T cells. The results demonstrated that ZAP70 and IRF4 did not show parallel expression patterns. Although IRF8 and IRF4 suggest that pairing of IRF8 and IRF4 is important, further studies are required to modulate these types of immune responses.

**053**

Beta arrestin-2 signalling attenuates contact allergic inflammation and inhibits proinflammatory chemokine production by keratinocytes

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Beta arrestin-2 (β-arrestin-2) is a G-protein-coupled receptor (GPCR) signaling mediator that is differentially expressed in various immune cell subsets. While β-arrestin-2 is constitutively expressed in all lymphocyte subpopulations, it is minimally expressed in neutrophils, monocytes, and macrophages. In contrast, β-arrestin-2 is highly expressed by T cells and is associated with the inhibition of Gαiq-dependent signaling, suggesting a role in the control of inflammatory responses. In this study, we investigated the role of β-arrestin-2 in the regulation of contact hypersensitivity responses. To this end, we performed real-time PCR using activated CD8+ T cells cultured in the presence of a ZAP70 inhibitor or a Jak3 inhibitor. The results demonstrated that ZAP70 and IRF4 did not show parallel expression patterns. Although IRF8 and IRF4 suggest that pairing of IRF8 and IRF4 is important, further studies are required to modulate these types of immune responses.

**054**

Time course effects of topical Dextran sulphate fumarate applications on the progression of atopic dermatitis-like symptoms in NC/Nga mice

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Atopic dermatitis (AD) is not a simple inflammatory skin disease but is a quite complicated skin syndrome influenced by genetic background and different types of environmental factors such as allergens and microbes. Although various animal models have been suggested to analyse the pathogenesis of AD and the development of therapeutic drugs for the disease, there are few reports to evaluate each stage of the skin condition in the course of AD progression. In this study, we investigated the correlation between the expressions of immunological factors and each stage of AD progression. To evaluate the time course effects of topical dextran sulphate fumarate applications on the onset and duration of AD-like symptoms, we applied Dextran sulphate fumarate extract (DSE) together with skin barrier disruption on the upper dorsal skin of NC/Nga mice twice a week for 8 weeks. Repeated application of DSE rapidly elevated the dextran score followed by histologic changes of the skin at each stage of AD progression in a time-dependent manner. From the 2nd week of the DSE application, the skin surface appeared the dryness and hemorrhage, and edema and exudation occurred from the 3rd week. Interestingly, we found that skin barrier disruption with 4% Sodium dodecyl sulphate (SDS) twice a week failed to develop the AD-like skin lesions in NC/Nga mice. We also identified the correlation between the expressions of AD-related immune regulatory factors, such as immunoglobulin (Ig) subclasses and cytokines, and the each stage of AD progression. The changes of the expressions of total and 17 cytokines affected the each stage of AD-like skin symptoms. In conclusion, we showed that the topical applications of DSE may be a useful tool for elucidating the pathogenesis of AD but also to develop and evaluate the therapeutic drugs for AD.
ABSTRACTS | Adaptive Immunity

055 Human Langerhans cells potently induce Th1 cytokines in the presence of poly(I:C), but are less responsive than dendritic cells towards bacterial stimuli.
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Recent studies report professional antigen presenting cells and provide a link between the innate and adaptive immune system. Langerhans cells (LC) represent a highly specialized subset of DC localized in the epidermis, an environment extensively exposed to pathogens. Due to their unique location it has been suggested, that resident LC are ideally positioned to capture invading viruses and possibly induce anti-viral immunity, but in contrast to dermal DC, contribute to tolerance towards bacterial commensals. Therefore, we analysed the maturation and cytokine production of human immature monocyte derived DC (MoDC) and LC like cells (MoLC) upon stimulation with TLR ligands, pro-inflammatory cytokines and soluble CD40 ligand (CD40L). MoDC were distinguished from MoLC, the expression of IC-Cathepin, Langerin and TROP2. Pro-inflammatory cytokines as well as CD40L highly induced the expression of co-stimulatory molecule CD86 and activation marker CD81 after 24h of activation. MoDC, representing dermal DC subsets, were strongly activated by bacterial signal, leading to upregulation of CD14 and CD69, together with production of IL-6, IL-10, IL-12p70, IL-23 and IFN-γ. In contrast, MoLC showed impaired responsiveness to bacterial TLR ligands, but highly matured in the presence of poly(I:C), a molecular pattern associated with viral activation from the antiviral-IFN-stimulated gene.

056 Effect of topical application of Quercetin on atopic dermatitis in N/CgNa mice

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Quercetin is a bioflavonoid that is present in a wide variety of fruits and vegetables. We previously demonstrated that topical application of quercetin (QRG) inhibits the expression of cyclooxygenase (COX)-2 and markedly decrease the levels of total plasma cytokines in C57BL6 mice. However, the potential role of QRG in vivo in the cutaneous inflammation remains unknown. The aim of this study was therefore to evaluate in murine model of atopic dermatitis (AD) the effect of QRG topical treatment to improve Df-induced AD-like inflammatory responses. These results demonstrate that QRG might be beneficial in the treatment of AD.

057 Induction of regulatory T cells by human antimicrobial peptides
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Antimicrobial peptides (AMP) are small molecules which are released by a variety of cells including keratinocytes. AMP were initially described according to their antimicrobial activity. They are essential components of the innate immune response and most responsible for antibacterial defense. Recently it was discovered that AMP exert additional activities beyond their antimicrobial capacities. They were demonstrated to influence the adaptive immune system by modulating antigen presenting cells. Recently we demonstrated that the murine beta-defensin-14 (mBD14) is able to induce regulatory T cells (Treg). mBD14-14 shifts to non-regulatory T cells into Treg by inducing the T cell receptor (TCR) and possibly inducing factor (i.a., IL-10) expression. Here, we investigated whether human AMP exert similar immunosuppressive features. For this purpose human peripheral blood mononuclear cells obtained from healthy volunteers were stimulated with different AMPs for 24h and the expression of CD103, perforin, granzyme B and PD-1 was assessed. CD83, a CD4+ T cell marker, 

058 Molecular characterization of ECP-induced monocyte-derived dendritic cells
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Extracorporeal photopheresis (ECP) in the treatment of cutaneous T-cell lymphoma, GVHD and autoimmune conditions continues to spur the question of how ECP is capable of inducing immunosuppression. We previously demonstrated that ECP treatment leads to large-scale conversion of peripheral blood monocytes into functionally competent foamy macrophages with dendritic cell phenotypes, which may play key roles in the immunomodulatory capabilities of ECP. To characterize this population of cells on a molecular level, we assessed for differential surface expression of selected gene-products on monocytes after treatment with a model-ECP apparatus. Four gene-products (CXCL16, SIRPa, ICAM1, TNFR1) showed significant increases in surface expression after model-ECP treatment as compared to PMCP (-p<0.01 for all). To identify transcription factors (TFs) expressed by ECP-treated monocytes but not peripheral blood monocytes, rPCR was performed. Interaction with platelets during ECP passage was also assessed by using model-ECP plates coated with low- and high-density platelets. Seven TFs demonstrated increases in mRNA after passage through the model-ECP plate (ARQ range: 1.35-7.6). Increased platelet density directed induction of mRNA expression (ARQ > 2.5) for VLA4, HSPA8, CD38, TNFR1, CD11c, HSPB1 and PTEN. ARQ of the transcription factor recently identified as being specifically expressed in classical DCs, demonstrated ~3-fold increase in expression after treatment. CREM, an important component of cAMP-mediated signal transduction and possible DC maturation marker, was significantly up-regulated with both platelet treatments (ARQ > 6.78 and 5.9 for low- and high-density platelets, respectively). In summary, passage through the ECP plate apparatus caused activation of novel surface molecules and transcription factors that define and characterize a unique subset of “physiologically-induced” DCs. The effects of these cells on the immunology of ECP treatment will be further elucidated.

059 CD8+ T cell epitopes in cutaneous malignancies
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Atopic Dermatitis (AD) is a chronic inflammatory skin disease that commonly begins in childhood. Quercetin-3-O-a-L-rhamnopyranosyl-2"-gallate (QRG), which was isolated from the bark of Acer ginnala Maxim green natively in Korea, has been known to have several biological effects, including anti-inflammatory and anti-cancer activities. In this study, we examined the effect of topical application of QRG on skin inflammation and AD-like skin lesions in mouse models. Over eight weeks, mice applied daily to QRG for 4 weeks received twice a week application of 100 mg Dermatophagoides farinae (DF) ointment on back for 4 weeks. Topical application of QRG were down-regulated the development of AD-like skin lesions in N/CgNa mice. Interestingly, QRG markedly decreased inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) mRNA expression in skin. QRG also significantly suppressed the level of total plasma Immunoglobulin (Ig) E induced by topical DF-stimulation. We also showed that topical application of QRG reduced the expression of PD-1 in skin biopsies of AD patients. In the present study, we demonstrate that topical application of QRG was able to improve Df-induced AD-like inflammatory responses. These results demonstrate that QRG might be beneficial in the treatment of AD.

060 The PD-1 pathway is a potential drug target for squamous cell carcinoma in solid organ transplant recipients

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Immunotherapies with anti-PD-1 and anti-PD-L1 mAbs have shown an impressive clinical response in patients with melanoma, and are currently being tested for other cancers. Squamous cell carcinoma (SCC) is a significant cause of mortality and morbidity in organ transplant recipients undergoing systemic cyclosporine A treatment. However, the involvement of the PD-1/PD-L1 pathway in these patients is unknown. We therefore sought to determine the expression of the PD-1 pathway in cutaneous SCC and the potential effect of cyclosporine A on this pathway. Immunohistochemical staining of PD-1 and its ligands (PD-L1 & PD-L2) was observed in the stromal region of SCC in immune competent patients (n=6) as well as in immune-suppressed transplant recipients (n=4), but not in normal skin of healthy volunteers (n=5). Double immune-fluorescent staining on SCC biopsies (n=3) showed the positive expression of PD-L1 on CD11c+ dermal dendritic cells, forming cellular clumps in the SCC stroma. In contrast, no double positive cells were found in normal skin (n=5). To determine the potential role of PD-L1 signaling, representative normal human keratinocytes (n=5) were treated with cyclosporine-A (100ng/mL) and induction of PD-L2 was observed at both 24h and 48h (p<0.05). In summary, we demonstrated the activation of the PD-1 axis in cutaneous SCC in transplant recipients as well as in normal keratinocytes. In addition, this study was important for mediating immune tolerance and inhibiting the immune response. Thus, the impact of cyclosporine-A on PD-L2 expression in keratinocytes may underlie the successful immune evasion mechanisms observed in transplant recipients. Although further functional analysis is required to elucidate the role of PD-L1 signaling associated with SCC progression, our data suggest that PD-1 is a potential target for immunotherapy in transplant recipients with SCC.

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Modulation of CD1d by hypersensitivity reaction inductor drugs.

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CD1d is a widely expressed cell-surface protein involved in many biological responses including the activation of the immune system and virus entry in the target cells. In B cells, CD1d is a component of the CD1d/CD21 co-receptor complex that allows cell activation and Epstein-Barr Virus (EBV) entry. EBV reactivation is frequently associated to drug reaction with eosinophilia and systemic symptoms (DRESS) which is a severe drug-induced hypersensitivity skin reaction. We previously showed that only the culprit drugs trigger the production of EBV in DRESS patients. Interestingly, recent studies show that CD1d overexpression could be modulated by drugs such as phenobarbital. Thus, we focused on the drug-induced modulation of CD1d protein expression. B lymphoblastoid cell lines from 5 DRESS patients and 6 healthy controls were incubated or not with sulfamethoxazole and valproic acid. However, there is no significant difference between DRESS patients and healthy controls. In conclusion, the identification of inductor drugs, such as sulfamethoxazole and valproic acid, and may facilitate viral entry in B cells.

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Genetic variations in IL6 and IL12B as protective markers for psoriasis.

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Recent studies associate a number of genetic polymorphisms to psoriasis. In this direction, we aimed to assess the protective potential of three genetic variants of interleukin genes. In the present case-control study we genotyped a group of 67 psoriasis patients and 69 healthy subjects for IL6 rs1800795, IL12B rs1212227 and IL12B rs6878695. All data was then statistically analyzed using SPSS. Statistical significance was present for IL6 rs1800795, IL12B rs6878695 (p<0.001, p<0.028, respectively). In the case-control analysis, IL6 rs1800795 CC vs. GG carrier showed a strong protection against the disease (OR=0.072, p<0.018), as did IL12B rs6878695 CC vs. GG carrier (OR=0.018, p<0.025). All these markers in a multivariate analysis increased the protective value to 95.5% for CC vs. GG in both IL6 rs1800795 and IL12B rs1212227 (p<0.006). Concluding, we report the minor alleles of IL6 rs1800795 and IL12B rs6878695 as potentially protective markers for psoriasis.
**ABSTRACTS | Adaptive Immunity**

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*The regulatory T cell population is altered in atopic dermatitis*  
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Atopic dermatitis (AD) is considered as a dermatologic disease resulting from skin barrier disorders and overreacting skin immune response. However, the role of immunosuppression and especially the relative regulatory T cells (Tregs) remains unclear. In this work, we aimed to characterize Tregs in AD patients and in two mouse models of AD. Adult patients with AD lesions showed elevated dermalization of CpG dinucleotides in a conserved region of FoxP3 intron 1 in peripheral blood, which demonstrates more Tregs expressing suppressive function as compared to healthy controls and non-lesional AD patients. Similarly, both mouse models i.e. topical treated with vitamin D3 (VitD3) or oespressing TSP under a K14 promoter, exhibited increased ICOS-expressing CD4+CD25+FoxP3+ Tregs in skin draining lymph nodes (sDLNs). Moreover, proportions of natural Tregs are increased compared to induced Tregs, suggesting that AD lesiongenesis, showed limited proliferation, direct suppression of Tregs by VitD3 was excluded by removal of the application site and instead, skin-derived dendritic cells (DCs) are required for Treg induction. Indeed, Langerhans cells (LCs) are the first skin-derived DC subset to reach the skin lesion and are important for tumor immune response targeted by all trans retinoic acid and vitamin D3. However, the roles of irritant-induced ROS in skin lesionogenesis, is not fully characterized. Previously, we have shown that allergen-induced ROS increase immune responses in X.S106 DCs and human monocyte-derived DCs (MoDCs). However, the roles of irritant-induced ROS in X.S106 DCs are not fully characterized. Herein, we have evaluated the roles of ROS produced in X.S106 DCs by irritant, benzalkonium chloride (BKC). ROS were produced by BKC in X.S106 DCs and ROS production was increased in correlation to the incubation time. When pretreated with GSH, catalase and vitamin E, ROS productions were blocked. Apoptosis was increased in BKC-treated X.S106 DCs in concentration-dependent manner. However, antioxidants did not prevent apoptosis which implies that apoptosis by BKC is not related to ROS produced by BKC stimulation. Cell-surface molecules, such as CD80, CD86 and CD40 were not expressed on BKC-treated X.S106 DCs with significant differences. Cytokine production from BKC-treated X.S106 DCs was checked by ELISA. IL-12 and IL-6 secretions were not increased in X.S106 DCs by BKC. TNF-α was produced in X.S106 DCs with significant differences compared to control X.S106 DCs. In summary, the role of BKC-induced ROS in X.S106 DCs is different from TNBS-induced ROS. BKC-induced ROS in X.S106 DCs do not have a role related to the immune response but probably only related to inflammation.
Efficacy of RG1-VLP vaccination against genital and cutaneous human papillomaviruses in vitro and in vivo

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Licensed human papillomavirus (HPV) vaccines, based on virus-like particles (VLP) self-assembled from major capsid protein L1, prevent infection with HPV16 and 18, which cause 70% of cervical carcinomas and a subset of other genital and ophthalmic cancers. However, they may not protect against infections with less prevalent mucosal or cutaneous HPV. In contrast immunizations with minor capsid protein L2 induce low-titer but potentially cross-neutralizing antibodies. We have previously generated chimeric RG1-VLP by genetic insertion of HPV16/18 amino acids 17-36 (RG1) within the DE-surface loop of HPV16 L1. Vaccinations induced cross-neutralizing antibodies against homologous HPV16, 31 and cutaneous HPV27/57/58/68, by pseudovirus-or native virus-based neutralization assays (tits 25-1,000). Boostable antibody titers over 1 year and ELISPOT assays demonstrated the induction of B-cell-memory and CTL responses. Using a mouse germline challenge model with RG1-VLP we demonstrated that RG1-VLP rapidly cleared VACV infection from distant skin, while the uninfected parabiont, although it parabiotically joined it to a normal mouse. After four weeks, parabionts were separated, rested for one month, and challenged with RG1-VLP. As expected, the normal mouse was protected and did not develop VACV infection, whereas the infected parabiont was protected and remained free of VACV. These findings demonstrate that RG1-VLP offers protection against cutaneous and mucosal HPV types.

Development and functional competence of dermal dendritic cells in the human fetus

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Dendritic cells (DCs) are critical regulators of immune responses to pathogens, tumours and vaccines and tolerance to self. Immunological competence in the human fetus is poorly understood with scant attention paid to the contribution of fetal DC network to tolerance and immunity. Our studies aim to map the development and functional maturity of DCs in the human fetal dermis and how this impacts immune competence. Flow cytometry analysis of fetal skin revealed the presence of three myeloid DC subsets characterised by the expression of CD141, CD14 and CD14 from 12 weeks of gestation. These subsets correspond phenotypically to the adult cutaneous DC complement, including CD141+ Langerhans cells (LC) and CD14+ DCs with their respective adult counterparts from skin and blood. These findings highlight the early establishment of a comprehensive cutaneous DC network in the human fetus. Ongoing studies are focused on dissecting their specific immunological functions and competence.

Developmental and functional competence of dermal dendritic cells in the human fetus

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Recent studies have shown that parabiosis experiments further show that dermal γδ T cells play an important role in establishing CHS response. We next generated dermal γδ T cell-deficient mice by bone marrow transfer with injection of neonatal thymocytes to irradiated mice. These mice were then sensitized and challenged with injection of neonatal thymocytes to irradiated mice. These mice were then sensitized and challenged with the antigen of interest. Upon activation, these dermal γδ T cells produce large amounts of IL-17 and IL-22. GPP+δ-GFP+ parabiosis experiments further showed that dermal γδ T cells are constantly circulating, whereas δT cells are non-recirculating. In order to explore their role in CHS, we first sensitized and challenged TCR β+ mice with 2,4-dinitrofluorobenzene (DNFB). A remarkable reduction of CHS responses in these parabiotic mice was observed, suggesting that γδ T cells play a pivotal role in CHS responses. We next generated dermal γδ T cell-deficient mice by bone marrow transfer with injection of neonatal thymocytes to irradiated mice. These mice were then sensitized and challenged with house dust mite (HDM) and CHS responses were measured. Our results showed that CHS responses in dermal γδ T cell-deficient mice was significantly lower than that of wild type mice, demonstrating dermal γδ T cells do participate in CHS responses. Furthermore, we depleted IL-17-producing γδ T cells by using neutralizing antibodies and found that CHS responses were also significantly reduced in these mice. Therefore, our data suggest that circulating IL-17-producing γδ T cells in dermis play an important role in establishing CHS response.
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**079**

IL-22 single-producing CD4+ T lymphocytes: Candidate effectector cells in acute cutaneous graft-versus-host-disease

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Graft-versus-host disease (GVHD) is the major clinical complication of allogeneic hematopoietic stem cell transplantation (HCT) and can present in an acute and chronic form. The question whether similar or different pathomechanisms are operative in acute (aGVHD) and chronic GVHD (cGVHD), has not yet been resolved. To address this issue, lesional skin biopsies were obtained from aGVHD (n=25) and cGVHD (n=16) patients. The cellular infiltrate was assayed by immunofluorescence staining, interleukin and chemokines were measured by real-time RT-PCR. Cytokine secretion profile of isolated T cells from collagenase-digested lesional skin biopsies were analysed by intra-cellular flow cytometry. While CD4+ and CD8+ T cells dominated the inflammatory infiltrate in both acute and cGVHD, the analysis of the quality of the T cell-mediated immune response revealed striking differences. In aGVHD lesions, there was a predominance of Th2 cytokines (IL-4, IL-13) and chemokines (CCL17, CCL22). In accordance with these findings, we observed an increased IL-4-producing T cells in aGVHD lesions. Remarkably, levels of IL-22 mRNA were highly increased in cGVHD lesions, but CD4+ and CD8+ T cells produced IL-22 purely, while CD4+ T cells were shown to be major sources of IL-22 in cGVHD lesions.

**080**

Global circulating CD4+ and CD8+ T cells are more differentiated in old melanoma patients compared to healthy controls

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Melanoma is highly immunogenic but disease progression occurs despite the presence of tumour-specific T cells. Conventional antigen exposure may drive the melanoma specific T cells to an end-stage, as is the case in some chronic virus infections such as CMV. It is particularly pertinent amongst the elderly where melanoma incidence and mortality are highest. This study sought to assess T cell differentiation in melanoma patients aged 65 years and above. Flow cytometry was used to analyse T cell differentiation (CD27 and CD45RA expression) and CMV T cell responses in PBMCs from 23 melanoma patients and age matched healthy controls. Compared to the controls, melanoma patients had an inverted CD4/CD8 ratio. In the CD4+ T cell compartment there was a significant decrease in naïve (CD27-CD45RA+) (p<0.005) CD4+ T cells that was accompanied by an increase in the central memory (CD27-CD45RA+) (p<0.005) and effector memory (CD27-CD45RA- (p<0.05) populations. Amongst the CD8+ T cells a reduction in central memory cells (p<0.05) and an increase in effector memory cells re-expressing CD45RA (CD27-CD45RA+) (p<0.05) was observed. These trends were more pronounced and likely to be a general phenomenon in the elderly. The data suggest that the melanoma specific T cells have undergone a functional differentiation path, a process which is partially reversible and could be exploited as a therapeutic target.

**081**

TIM-3 does not act as a receptor for galectin-9

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TIM-3 is up-regulated in exhausted murine and human T cells and TIM-3 blockade was described to restore the function of these T cells. Here we show that the activation of human T cells is not affected by galectin-9. In a T cell model, there was a predominance of Th2 cytokines (IL-4, IL-13) and chemokines (CCL17, CCL22). In accordance with these findings, we observed an increased IL-4-producing T cells in aGVHD lesions. Remarkably, levels of IL-22 mRNA were highly increased in cGVHD lesions, but CD4+ and CD8+ T cells produced IL-22 purely, while CD4+ T cells were shown to be major sources of IL-22 in cGVHD lesions.

**082**

DEC-205 targeted nanoparticles for improved DC-borne antigen delivery and cross-presentation

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Dendritic cell (DC) transport of antigens is controlled by the resident or cDC in the lymph node.

**083**

Subcutaneous immunization to HIV gag is potentiated by Flt3L and develops through classical rather than migratory dendritic cells

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Alitretinoin, a retinoid binding to both RAR and RXR, demonstrated significant efficacy in the treatment of cutaneous T cell lymphoma, but the mechanism of action is not known. In this study, we analyzed structural cells as well as leukocyte subsets and performed a clinical study determining the skin-homing phenotype and activation status of leukocytes before and under treatment with alitretinoin. In direct comparison with the RAR against actin, alitretinoin showed markedly enhanced suppression of chemokine production in keratinocytes as well as inhibition of dendritic cell maturation and activation. Moreover, the T cell-activating capacity of alitretinoin-treated dendritic cells was significantly lower than those treated with actin. In vivo, patients undergoing alitretinoin treatment showed a marked downregulation of the “skin-homing” abilities of effector T cells. Furthermore, mixed leukocyte reactions in patients were significantly reduced during alitretinoin treatment. The results indicate that alitretinoin targets dendritic cells in vivo and in vitro to activate alitretinoin to DC with high efficiency and with the possibility of simultaneously targeting virtually any DC stimulatory motif.

**084**

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

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There have been several reports that the dual RAR and RXR agonist alitretinoin is able to reprogram effector T cells and dendritic cells. We investigated the effects of alitretinoin on Th1 cells, Th2 cells, Tregs and dendritic cells (DC) in vitro. The results suggest that the dual RAR and RXR agonist alitretinoin is superior to the RAR agonist actin in modulating skin inflammation.

**S14**

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085
Granzyme B plays a central role in delineating subsets and functional differences in human Tregs. In order to explore the effect of the gene on the consequent Treg function, we performed a pilot study with 10 healthy human donors, 5 males and 5 females. We isolated peripheral blood mononuclear cells (PBMCs) from the blood samples by centrifugation on Ficoll and determined the proportions of CD4+CD25+FoxP3+ Tregs by flow cytometry. We found that the proportions of Tregs were not significantly different between males and females, but were significantly lower in patients with a history of autoimmune disease (P < 0.05). These results suggest that the expression of Granzyme B may be modulated by factors such as sex and disease status, which may play a role in the regulation of Treg function.

086
Impaired CCR7-mediated migration of MUTZ-3-derived Langerhans-like cells in response to interferon-gamma and recombinant stimulants
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Epidermal dendritic cells (Langerhans cells (LC)) are recognized to play an essential role as antigen-presenting cells in the skin. They are involved in the initiation of immune responses against skin pathogens and skin tumors. The migration of LCs to sentinel lymph nodes (SLNs) is crucial for the induction of effective immune responses. The expression of CCR7, a chemokine receptor, is essential for the migration of LCs to lymph nodes. In this study, we investigated the expression and function of CCR7 in MUTZ-3-derived LCs in response to interferon-gamma (IFN-γ) and recombinant stimulants. We found that IFN-γ and recombinant stimulants impaired the migration of MUTZ-3-derived LCs to lymph nodes in vitro. These results suggest that IFN-γ and recombinant stimulants may impair the induction of effective immune responses against skin pathogens and skin tumors.

087
Assessment of nickel sensitivity using a T-cell mediated immune response assay
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We conducted a pilot investigation of an adenosine triphosphate (ATP) based in-vitro assay for diagnosing nickel sensitivity, one of the most common causes of contact allergy. A T-cell mediated immune response assay (Cylex Inc., Columbia, Maryland) was used to detect nickel-sensitive T cells. Whole blood was added to a microtiter well and incubated with Con A and nickel in CD3 lymphocytes. A total of 8 individuals were enrolled following patch testing. Patients were tested with nickel and controls to determine a dose-response curve. Following incubation for 15 to 18 hours in a 37°C, 5% CO2 incubator, CD3+ cells were isolated using magnetic particles coated with anti-human CD3 monoclonal antibody, washed to remove residual cells and seeded into a microtiter well containing a fluorescent ATP marker. The reaction was visualized using a fluorescence activated cell sorter. The response was measured as the fluorescence of the ATP marker. Optimum responses were seen with 25 and 50 microM solutions. ATP values did not follow specific trends in controls. Limiting factors of this study are the small cohorts, limited number of lymphocytes per milliliter and using a non-specific marker (CD3) for T cells. Our results suggest that this assay is a promising tool for diagnosing nickel sensitivity, but further studies are needed to optimize the assay and validate its clinical utility.

088
Genetic or chemical RORγt blockade profoundly suppresses hapten mediated contact hyper-sensitivity responses
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We used a functional approach to investigate the role of RORγt in mediating contact hypersensitivity (CHS) responses. We generated RORγt(-/-) and control mice and performed CHS reactions to nickel. As expected, RORγt(-/-) mice showed a significant decrease in CHS response compared to control mice. We further investigated the role of RORγt in mediating CHS responses by using specific RORγt agonists and antagonists. We found that RORγt agonists significantly suppressed CHS responses, while RORγt antagonists had no effect. These results suggest that RORγt plays a crucial role in mediating CHS responses.

089
Melanoma-silencing through CD4+ T helper (Th1) cell cytokine-induced senescence
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Adaptive T-cell transfer is a promising therapeutic option for patients with metastatic malignant melanomas. Recent evidence suggests that CD4+ Th1 cells and Th1 cytokines are of great importance for the induction of immune responses against melanoma. In this study, we investigated the effect of human melanoma-specific CD4+ Th1 cells and Th1 cytokines on melanoma cells. We generated melanoma-specific CD4+ Th1 cells by adoptive transfer of melanoma reactive T cells into normal mice. We then co-cultured melanoma cells with Th1 cells and Th1 cytokines. We found that Th1 cells and Th1 cytokines induced senescence in melanoma cells, as evidenced by stable growth arrest in vivo. These results suggest that Th1 cells and Th1 cytokines have the potential to induce immune responses against melanoma.

090
Tissue resident and central memory T cells share a single clonal origin but play distinct and non-overlapping roles in long-term memory responses in skin
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Tissue resident memory T cells (T RM) provide rapid and effective anti-viral protection, superior to that of circulating central memory T cells (TCM). In our latest work, this emerging paradigm shift in understanding immune memory can now be extended to non-viral antigens. We used functional in vivo and in vitro assays, parabiotic mice, and high throughput TCR sequencing, to show that populations of long lived T RM cells mediate a rapid response to contact sensitizers (e.g., DNFB). In normal mice, the local skin response of sensitized mice to DNFB is ultra-fast, site specific and involves TCM and T RM cells. However, in parabiotic mice, TCM cells are not detected in the skin and T RM cells are increased. This suggests that T RM cells are generated in the skin and provide a rapid response to contact sensitizers. These results highlight the importance of T RM cells in mediating long-term memory responses in skin.

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**091**

**Systems analysis of pneumococcal vaccine response in psoriatic patients**


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“Exercising” the immune system by vaccination allows to dynamically investigate the human immune system in an ethically acceptable manner and make immune alterations, not visible at steady state, measurable. We have investigated patients with chronic plaque type psoriasis receiving either inactivated (n=15), which simultaneously blocks the IL-12 and IL-23 axis, or who were untreated with systemic immunosuppressants or biologics for a minimum of 8 weeks (n=18). Untreated patients were selected to match demographics and PASI scores of treated patients (median age 41 years, median PASI 3.4). Patients received the 23-valent pneumococcal polysaccharide vaccine. Using systems approaches, we collected in addition to detailed clinical data and complete blood counts also data from biopsies for whole blood mRNA, fresh whole blood 10-color polyphenolic flow cytometry and serology. Measuring binding antibodies to 14 polysaccharides, no significant difference in serological response between psoriasis patients without systemic treatment or receiving methylprednisolone, or a biologic, auto group (n=12) was noted. The quality of the antibody response (agonizing antibodies titers) will be further investigated. While a spike in circulating CD19+CD22+CD17+CD198 plasmablasts was observed on day 7 following vaccine administration in 36% of patients, no increase in CD107a expression was observed on naive CD8+ T cells by day 28 post-vaccination. DC from CCR7-deficient hosts and purified CD8+ DCs (a mouse equivalent to the human LC are the two mouse thymic DC subsets. Our studies also revealed a distinct genetic signature of over 25 genes, that could identify and predict which DC subsets can cross-present. The cross-presentation signature was present in the human blood CD141+ DCs and correctly identified the ability of other distinct human and mouse DC subsets to cross-present. The cross-presentation signature was present in the human blood CD141+ DCs and correctly identified the ability of other distinct human and mouse DC subsets to cross-present.

**092**

**Induction of a long-lived protective mucosal T cell response by inactivated Chlamydia trachomatis coupled to a TLR7 agonist by nanoparticles**


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The obligate intracellular pathogen Chlamydia trachomatis (Ct) is the most commonly reported sexually transmitted bacterial infection and the world’s leading cause of infectious blindness and female infertility. We designed a vaccine consisting of inactivated Ct and a TLR7 agonist covalently linked to nanoparticles that were attached to the microbial surface and, using a mouse model of Chlamydia-induced hair loss. T cells are considered the critical pathogenic cells, since they abundantly infiltrate the dermal Langerhans cells (LCs) were highly efficient at priming effector CD8+ T cell responses. How- ever, the ability of mouse LCs to cross-present antigens and drive potent CTL responses is controversial. We used expression profiling to find the human LC equivalent in the mouse. Our studies suggest that the mouse equivalent to the human LC are the two mouse thymic DC subsets. Our studies also revealed a distinct genetic signature of over 25 genes, that could identify and predict which DC subsets can cross-present. The cross-presentation signature was present in the human blood CD141+ DCs and correctly identified the ability of other distinct human and mouse DC subsets to cross-present.

**093**

**Diverse cutaneous dendritic cell subsets play distinct roles in the transport of soluble pro- teins to draining nodes and subsequent cross-presentation to naïve CD8 T cells**


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We have developed a system in which dendritic cells (DC) isolated from the skin-draining lymph nodes of cutaneously immunized mice present their in vivo-acquired antigen to naïve CD8 T cells ex vivo. Whole body photoacid was used as the topical immunization, as it requires processing within DC to cross-prime naive ovalbumin-specific CD8 T cells. DC populations from immunized wildtype (WT) mice stimulated robust proliferation of OT-I T cells. This system has allowed us to explore the relative contribution of various DC subsets and DC-expressed molecules to the process by which cutaneous antigen is delivered to antigen-specific naïve CD8 T cells. We used various mouse breeding and cell sorting techniques to acquire greatly enriched populations of several distinct DC subsets. DC populations from immunized CCR7-deficient mice were unable to stimulate OT-I proliferation, suggesting that migratory DC are required for cross-presentation. DC populations from immunized WT mice resident DCs had OT-I+ CD8+ T cells. DCs from immunized WT mice were capable of presenting both CXCR3+ and CXCR5+ CD8+ T cells. In summary, we here show that although changes in immune cell composition in the blood may not be visible in psoriasis patients with mild disease activity in the steady state, such alterations can become apparent when “exercising” the immune system by vaccination.

**094**

**Transcriptional and functional conservation between human Langerhans cells and mouse thymic DCs**

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The characterization of human DC subsets is essential for the design of new vaccines and for better translation of mouse immunological data to the clinic. Previously, we showed that human epidermal Langerhans cells (LCs) were highly efficient at priming effector CD8+ T cell responses. However, the ability of mouse LCs to cross-present antigens and drive potent CTL responses is controversial. We used expression profiling to find the human LC equivalent in the mouse. Our studies suggest that the mouse equivalent to the human LC are the two mouse thymic DC subsets. Our studies also revealed a distinct genetic signature of over 25 genes, that could identify and predict which DC subsets can cross-present. The cross-presentation signature was present in the human blood CD141+ DCs and correctly identified the ability of other distinct human and mouse DC subsets to cross-present.

**095**

**Longitudinal characterization of an antigen-specific T cell response**

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During the primary immune response antigen-specific T cells are activated and T cell proliferation and differentiation occurs. Competition among different T cells specific for the same antigen helps shape the T cell response. Following antigen clearance the immune response wanes and T cells under apoptosis. However, a memory response remains. These memory T cells are poised to respond quickly and efficiently to antigen rechallenge. In general, it is believed that the most high affinity clones are selected to become components of the memory pool. Using T cell repertoire chain sequencing, we have identified strikingly similar repertoire is regenerated following myeloablation and syngeneic bone marrow transplantation. Finally, we demonstrated that in, healthy animals, a profound repertoire shift can be observed in the secondary immune response following antigen rechallenge. This repertoire shift depends on the conditions used to prime the animals. In contrast to current dogma, we observed that high affinity antigen-specific CD8+ T cells are transiently lost during the secondary immune response, even though they are well-represented in the primary immune response.

**096**

**Next generation T cell receptor sequencing for the identification and monitoring of patho- genic T cell clones in alopecia areata**


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Alopecia Areata (AA) is one of the most common autoimmune diseases, characterized by non-scarring hair loss. T cells are considered the critical pathogenic cells, since they abundantly infiltrate the hair follicles in AA, and are both necessary and sufficient to disease in the C3H/HeJ mouse model of AA. Next generation T cell receptor (TCR) sequencing provides a platform to iden- tify and track the frequency of pathogenic TCR clones, representing a functional biomarker of disease activity. Using this technique for TCR beta chain sequencing, we have identified strikingly expanded T cell clones in lesional skin from five AA patients, which represented up to 8.5% of the total TCR sequences, supporting an antigen-driven process in AA, and providing evidence of dominant pathogenic TCR clones. Comparative data from the C3H/HeJ mouse model of AA also strongly supports this notion. Indeed, as identical TCR sequences were dramatically expanded in new AA lesions of recipient mice grafted with lesional skin from the same donor. In human AA patients, we found that some T cell clones that were expanded in affected skin, also circulate at significant frequencies (>0.1%) of total blood sequences in the peripheral blood of the patient. By correlating the circulating CD8+ T cell subsets to the frequencies of circulating pathogenic clones, we are now testing the hypothesis that circulating pathogenic TCR frequency distinguishes patchy Alopecia (AAP) and generalized Alopecia (AG), and correlates with baseline disease severity or disease trajectory. In a longitudinal study, we will determine if increased circulating pathogenic TCR frequency precedes or occurs concomitantly with disease progression. The unique accessibility of clonally expanded pathogenic T cells within the hair follicle end organ provides the opportunity for the development of new tools to monitor disease progression and the applicability of next generation sequencing to identify and track pathogenic TCR clonotypes in human autoimmune.