001 Relative roles and functional relationships of resident memory T cells (T RM) and central memory T cells (T CM) generated in response to skin antigenic challenge

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We recently demonstrated that skin TRMs provide much more rapid and effective anti-viral protection than do circulating TCM specific for the same infectious antigen. In the present study, we studied non-infectious antigen challenges to further assess the role of these two memory T cell populations. We demonstrated that chemicals that mediate contact hypersensitivity induces a population of skin resident TRM that mediate a very rapid recall response. This response is stronger at the site of sensitization, but also occurs at distant skin sites. We used para-biotic mice to further study this phenomenon, and demonstrate that contact sensitized antigen-specific blood TCM equilibrate between mice, while skin TRM do not. Using this model, we show that populations of antigen specific T cells that are circulating are generated concomitantly during sensitization. We find that skin TRM mediate rapid (<24h) recall responses, but that in their absence, TCM can infiltrate the skin and mount a slower immune response (>72 hrs). We next asked whether TRM and TCM can be derived from the same naïve T cell pool. Using highly-photoblot TCM sequencing, we assessed the T cell clonal repertoire response to DNBC, and in parallel to ovalbumin and to modified Vaccinia Ankara virus, and followed these clones molecularly in normal and para-biotic mouse models. We find that T cells carrying the identical TCR CDR3 Vb sequence—thus originating from the same naïve cell clonal repertoire response to DNBC, and in parallel to ovalbumin and to modified Vaccinia Ankara virus, exist in both TCM and skin TRMs.

002 Complete cutaneous B-cell lymphoma (A20) regression following intratumoral injections of A20-specific TCM effector (R9-Grim19) alone or combined with anti-CD40 and anti-CD28 costsimulation of IL-10-producing into IL-17/IFN-gamma-producing phenotypes

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To inhibit the STAT3-mediated signaling cascade in cancer microenvironment, we generated a fusion protein, designated R9-Grim19, that contained two distinct components—the non-agonistic protein-transduction domain (R9-PTD), and Grim-19 protein—because Grim-19 physically interacts with STAT3 and IP10/TT12 are transduced efficiently when injected in tumors in vivo. Our previous results demonstrated that intratumoral (i.t.) injections of R9-Grim19 alone elicited complete regression of established A20 tumor (B-cell lymphoma line with phosphorylated STAT3, pSTAT3 expression and IL-10 production, but no IL-4) in mice by eliciting A20-specific CTL activity. Based on these results, we investigated how immunocompetent cells participated in antitumor effects. Our results demonstrated that R9-Grim19-mediated antitumor effects were not observed in A20-bearing nude mice, but those were restored simply by the adoptive transfer of normal CD3+ T cells. Although pSTAT3 expression was elevated in all CD4+ and CD8+ T cells in tumor-draining lymph nodes (DLN) of untreated A20-bearing mice with IL-10-producing phenotypes, pSTAT3 expression was completely reverted to basal levels with dramatic conversion into IL-17/IFN-gamma-producing CD4+ and CD8+ T cells in both spleen and DLNs after i.t. injections of R9-Grim19. Interestingly, many CD4+ and FoxP3+ cells infiltrated the A20-tumor sites in mice treated with i.t. injections of R9-Grim19. These results indicated that local administration of R9-Grim19 had some advantages as a novel anti-STAT3 cancer immunotherapy because it strongly inhibited STAT3-regulated molecules in cancer microenvironment, including IL-10.

003 Regulatory T cells suppress basophil-induced IgE-mediated chronic allergic inflammation

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Regulatory T cells (Tregs) play important roles not only in the maintenance of skin homeostasis but also in the regulation of immune responses through suppressing the effector T cell functions in the skin. However, it remains to be clarified whether Tregs are capable of modulating basophilic functions. To determine whether Tregs influence basophilic functions, we studied IgE-mediated chronic allergic inflammation induced by the activation of basophils. Here we found that depletion of Tregs by anti-human CD25 antibodies, using human CD25/Fc region-smeared mice (FACS-sorted TCM) resulted in delayed bone marrow-derived basophils from 1194 GT2RFP reporter mice cocultured with Tregs exhibited decreased percentage of IL-4/IL-13 producing basophils. Notably, adoptive transfer of Tregs normalized the enhanced basophil numbers in the skin lesion of mice chronically sensitized to mite antigens, continuing the lack of basophils in bone marrow and basophilic numbers in both skin and ear swellings and histological features, including eosinophil and neutrophil infiltration. Thus, Tregs are critical for termination of the basophil-dependent chronic allergic inflammation.

004 Mast cells establish effective anti-tumor immune defense

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Despite detectable tumor-specific T cells in cancer patients immune defense fails to reject the tumors in most cases. However, in cutaneous melanoma, T cell mediated tumor regression is frequently observed and we identified high numbers of mast cells (MC) especially within the area of tumor regression. We hypothesized that accumulated MC otherwise supporting tumor development and growth may orchestrate cancer defense once activated by the appropriate danger signals. One important receptor for danger signals is Toll-like receptor 4 (TLR4) and in vitro TLR4 ligation resulted in potent activation of human and mouse MC. Consequently, we analyzed a murine model of cutaneous melanoma. After one week of tumor growth, when MC are already recruited to the melanomas, activation of TLR4 significantly enhanced T cell recruitment to the melanomas and tumor defense in wildtype but not in mast cell deficient sash mice. In this model TLR4 ligands selectively target MC because reconstitution of TLR4-/- mice with wildtype MC but not with TLR4-/- MC established tumor regression. We hypothesized that MC participate in anti-tumor immune defense by secreting soluble factors. Exosomes which are small membrane vesicles of endocytotic origin are thought to play an important role in intercellular communication. Therefore, we investigated immune effects induced by keratinocytes’ exosomes. First, we analyzed the release of different immune mediators from keratinocytes’ exosomes in different inflammatory conditions (+/- IFN gamma) and in the presence or absence of TLR4 and TLR9. During inflammation, keratinocytes not only function as mechanical barrier but also influence immune processes by secretion of soluble factors. Exosomes which are small membrane vesicles of endocytotic origin are thought to play an important role in intercellular communication. In this study, we show that keratinocytes produce exosomes and that these exosomes have anti-tumor immune effects upon TLR4 activation confirming the crucial role of MC and MC derived IP-10 for effective tumor defense. Searching for underlying mechanisms we identified a selective marked upregulation and secretion of the chemokine IP-10 (XCL1) following TLR4 mediated MC activation. By using IP-10 deficient MC we showed that IP-10 is important for effective tumor defense. Searching for underlying mechanisms we identified a selective marked upregulation and secretion of the chemokine IP-10 (XCL1) following TLR4 mediated MC activation. By using IP-10 deficient MC we showed that IP-10 is important for effective tumor defense

005 Dermcidin deficient mice initiate the elicitation phase of contact hypersensitivity via immunological synapse formation

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Allergic contact dermatitis is a common skin disease, characterized as a localized rash induced by coin-toss with a foreign antigen. Contact hypersensitivity (CHS) is a murine contact dermatitis model, and consists of the sensitization and elicitation phases. Thus far, the roles of cutaneous dendritic cells (DCs) in the sensitization phase have been extensively studied, however, their roles in the elicitation phase remain largely unknown. Initially, we evaluated the antigen presenting capacity of cutaneous DCs by DC subset-specific deletion system using CD11c-ΔDTR (diphtheria toxin receptor and Langerin-ΔDTR mice in combination with bone marrow chimera techniques. Depletion of LCs alone in the elicitation phase did not change CHS responses 24 h after elicitation, while depletion of dermal DCs alone impaired CHS. These findings suggest that dermal DCs are essential for the establishment of CHS. Next, we analyzed the dynamics of dermal DCs and memory T cells in the elicitation phase of CHS using a two-photon microscopy system. We found that dermal DCs actively migrated and accumulated in perivascular areas of the dermis after elicitation, and dermal DCs and memory T cells interact for several hours. This interaction was abrogated by anti-IFN-g neutralizing antibody treatment in vivo in accord with attenuated memory T cell proliferation in situ and impaired CHS responses. Taken together, our current findings suggest that dermal DCs are responsible for antigen presentation in the elicitation phase and that memory T cells interact with DCs for local T cell proliferation and establishment of CHS in an IL-1A-dependent manner, which is similar to immunological synapse formation in the lymph nodes in the sensitization phase of CHS.

006 Immunostimulatory effects of exosomes from keratinocytes

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Keratinocytes are a major component of the skin, keratinocytes not only function as mechanical barrier but also influence immune processes by secretion of soluble factors. Exosomes which are small membrane vesicles of endocytotic origin are thought to play an important role in intercellular communication. However, it remains unknown whether keratinocytes produce exosomes as immune modulators. Thus, we investigated immune effects induced by keratinocytes’ exosomes. First, we analyzed the release of exosomes from a murine keratinocyte cell line (MEPIK), as well as their composition by multiple approaches (ultracentrifugation, electron microscopy, western blot, and proteomics). Keratinocytes produced exosomes under steady-state as well as different inflammatory conditions (+/- IFN-g). Further, we examined the uptake of these exosomes by dendritic cells (BMDCs) and their effects on phenotypic and functional maturation. BMDCs showed a more mature phenotype indicated by a significant increase of both CD40 positive cells and CD40 antigen expression as well as the production of large amounts of IL-12, 10, and 12A cytokines. Furthermore, we explored whether these exosomes were able to transfer antigen-specific information from keratinocytes over BMDCs to T cells by inducing antigen-specific T cell stimulation of CD4 or CD8 T cells from C57BL/6 or BALB/c mice, respectively. Keratinocytes took antigen (ovalbumin) which was also present in exosomes as detected by western blot. However, exosomes failed to transfer antigen to BMDCs in substantial amounts to induce antigen-specific T cell proliferation. Together, these findings suggest that keratinocytes produce exosomes which are important in intercellular communication and mediate rapid (<24h) recall responses under different inflammatory conditions by secreting differentially loaded exosomes.
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007 Complete B16 melanoma regression with Trp2-specific CTL expansion is elicited by inattenuated ovalbumin vaccination in addition of a novel STAT3 inhibitor (9R-GR90149) plus Tc1/Tc17 inducer without melanoma-specific immunotherapy

S Hanawa, T Oshikado and S Shima. Department of Dermatology, University of Yamanashi, Yamanashi, Japan. The cancer microenvironment severely limits the efficacy of various immunotherapies in part through phosphorylated STAT3 (pSTAT3). In culture, we confirmed that IL-4 and IL-10 secreted from B16 cells with an attenuated ovalbumin (AOB) vaccine, drastically suppresses STAT3 activation. Interestingly, pSTAT3 expression in all CD4+ and CD8+ T cells of tumor-draining lymph nodes (DLNs) was elevated on day 5 after subcutaneous transplanted B16 melanoma. Although intratumoral (i.t.) injections of R9 protein transduction domain (PTD)-containing GR90149 (9R-GR90149; a novel STAT3 inhibitor, alone elicited anti-B16 effects, complete B16 regression or IFN-gamma-producing T cells were not observed. Previously, we also demonstrated that i.t. immunization with R9-PTD-containing AOB fusion protein (R9-AOB) elicited anti-B16 melanoma effects with presenting OVA-epitopes onto B16 cells at injection area and with OA-specific Tc1/Tc17 immune responses. However, anti-B16 effects by 9R-AOB were so severely diminished when those immune manipulation was stopped and after 5 days. To elicit complete B16 regression, we investigated the antitumor effects by various combined immunotherapies with 9R-GR90149. 9R-GR90149 elicited the enhanced antitumor effects when combined either with 9R or AO-AOB, drastically contrary to our previous observation. The strong memory effects by complete B16 rejection after rechallenge. These results indicate that B16-specific CTL expansion and antitumor effects could be elicited without any specific immunotherapies when optimizing the cancer microenvironment. These results remind us the reconstitution of current cancer immunotherapies.

009 CB1 receptor deletion specifically on keratinocytes leads to enhanced IFN-gamma-dependent secretion of proinflammatory cytokines and increased allergic contact hypersensitivity

S. Hanakawa, M. Kodke, T. Baldi, R. Kuner, A. Zielinski, 1 Dept. of Dermatology and Allergology, University of Boorn, Boorn, Germany, 2 Institute for Pharmacology, University of Heidelberg, Heidelberg, Germany, 3 Institute of Molecular Psychiwy, University of Bonn, Bonn, Germany and 4 Institute of Physiological Chemistry, University of Mainz, Mainz, Germany. Epidermal keratinocytes (KC) and cannabinoid (CB) receptors both participate in the regulation of inflammatory responses in a mouse model for allergic contact dermatitis, the contact hypersensitivity (CHS) response to the obligate sensitizer 2,4-dinitrofluorobenzene (DNFB). We investigated the cellular and molecular mechanisms how CB1 receptor, attenuates CHS responses to DNFB. For this purpose we used a conditional gene targeting approach to identify the relative contribution of CB1 receptors on epidermal keratinocytes for the control of CHS responses. To determine the underlying mechanisms that regulate these inflammatory responses in the effector phase of CHS, we performed further investigations on inflammation ear tissue and primary keratinocyte cultures using morphologic, molecular and immunologic methods. Mice with a keratinocyte-specific deletion of CB1 receptor developed increased and prolonged CHS responses. These were associated with enhanced reactive epidermal acanthosis and inflammatory keratocyte hyperpolarization in the effector phase of CHS. In vivo, primary cultures of CB1 receptor-deficient KC released increased amounts of TNFα and IL-10 and CC chemokines following stimulation with IFNγ compared to controls. In vivo, contact allergic ear tissue of CB1 receptor-deficient keratinocytes showed enhanced expression of CCL10 and CCL2 compared to controls. Further investigations establish CB1 as a proinflammatory mediator in the regulation of the immune response in the effector phase of CHS. Therefore, we conclude that CB1 receptors are functionally expressed on keratinocytes in vivo and help to balance the secretion of proinflammatory cytokines which regulate T-cell-dependent inflammation in the effector phase of CHS.

010 Pregnant X Receptor (PXR) modulates CCRL2 and Langerhans cell migration via TGF-beta1

A Eiter, M Schmidt, M赫尔曼, F Gonzalez and S Fuhlen. 1 Department of Dermatology, Medizinische Universität Innsbruck, Innsbruck, Austria, 2 Department of Internal Medicine, applaud, Innsbruck, Austria and 3 Laboratory of Immunology, Medical University of Vienna, Vienna, Austria. The pregnane X receptor (PXR) is a ligand-activated transcription factor regulating genes central to drug and hormone metabolism in the liver. We here show that PXR is highly expressed in different subsets of mouse and human immune cells especially Langerhans cells (LC). In addition down-regulated in mature DC. PXR ligands (PCN, pregnenolone, RU486, hydroxy- or methoxy-4-cholesteryl-acetate) dose-dependently down-regulated expression of CCRL2 and enhanced LC migration. We found that 65.3% of CD45+ leukocytes exhibit the scavenger receptor CD36 at 9 weeks estimated gestational age. Strikingly, significantly fewer CD45+CD14+ cells in embryonic skin express CD36 compared to adult skin (18.4% vs. 88.3%, p<0.001). CD45+CD14+ cells are located predominantly in the dermis, whereas subsets of CD16+ leukocytes can be identified with regard to the expression of CD14 and HLADR. Of note, CD45+CD16+ cells are occasionally also observed in the embryonic epidermis. While a subset of these epidermal leukocytes coexpresses CD14 and CD16, they do not express CD36. Leukocytes in various mouse models our data suggest that immature skin leukocytes acquire HLADR during development in the skin. In addition, we found that CD14+CD16+ leukocytes are present in the developing epidermis underscores the potential role which has been attributed to various putative, already committed CD14+ LC precursors in adult skin.

011 Possible regulatory roles of T regulatory cells in the development of atopic dermatitis-like Th2-dominant cutaneous inflammation induced by repeated epicutaneous hapten application

S. Hanakawa, A Kitoh, M Sugita, Y Myachii and K Kabashima. 1 Department of Dermatology, Graduate School of Medicine, Kyoto university, Kyoto, Japan and 2 Laboratory of Cell Regulation, Institute for Virus Research, Kyoto university, Kyoto, Japan. Atopic dermatitis (AD) is characterized by cutaneous hyperreactivity to environmental triggers. These long-lasting immune responses are associated with chronic inflammation, which results in the development of pruritic skin lesions. The pathogenesis of AD is not yet clear; however, regulatory T (Treg)-associated genes revealed Th2 and Treg-dominant cutaneous cytokine milieu in AD patients. Atopic dermatitis (AD) is characterized by cutaneous hyperreactivity to environmental triggers. These long-lasting immune responses are associated with chronic inflammation, which results in the development of pruritic skin lesions. The pathogenesis of AD is not yet clear; however, regulatory T (Treg)-associated genes revealed Th2 and Treg-dominant cutaneous cytokine milieu in AD patients. In AD, T cells show a pro-inflammatory phenotype that is characteristic of Th2 and Treg cell subsets, although the precise role of these cells in the pathogenesis of AD remains unclear. We hypothesized that Th2 and Treg cells play a role in the regulation of AD, and we investigated the regulatory role of Treg cells using Foxp3-GFP reporter mice, which allow selective depletion of FOXP3+ Treg cells. In the present study, we aimed to clarify the role of Treg cells in the pathogenesis of AD. We first investigated the role of Treg cells in the pathogenesis of AD using Foxp3-GFP reporter mice. We found that the depletion of FOXP3+ Treg cells resulted in a Th2-dominant cutaneous inflammation, which is characterized by increased Th2 cytokine production and reduced expression of Th1 cytokines. These results suggest that Treg cells play a role in the regulation of Th2-dominant cutaneous inflammation in AD. Our findings provide novel insights into the role of Treg cells in the pathogenesis of AD and suggest that targeting Treg cells may offer a new therapeutic strategy for the treatment of AD.

012 Distinct differentiation requirements and functional properties of mouse-specific human TH17 cells

C. Zielinski, A Landesczek, D Jamieson, F Ronchi and F Sallusto. 1 Department of Immunology and Allergology, CHU Bordeaux, Bordeaux, France and 2 Cellular Immunology, Institute for Research in Biomedicine, Bellinzona, Switzerland. Th17 cells have emerged as a new T helper cell lineage involved in the clearance of extracellular parasites and fungi. A dysregulated Th17 response, however, can induce severe tissue destruction and autoimmunity. Therefore, mechanisms must be in place to shield the host from immune-mediated damage. We demonstrate that human Th17 cells transiently produce the anti-inflammatory cytokine IL-10 upon stimulation. Interestingly, IL-10 expression was accompanied by reciprocal down-regulation of IL-17, leading to a functional regulatory Th17 cell phenotype after the peak of the effector response. The ability of Th17 cells to express IL-10 was, however, restricted to certain antigenic specificities. Ex vivo isolated, allogeneic-specific Th17 cells could not produce IL-10 in comparison to human-specific specific Th17 cells. This was due to differential priming requirements of these Th17 cell subpopulations. IL-17A-instructed naive T cells develop into a pro-inflammatory non-IL-10 expressing Th17 cell subset. Th17 cell priming with S. aureus, however, was IL-10 dependent, leading instead to the generation of IL-17 producing Th17 cells with self-regulatory activities. We investigated the development of Th17 cells under in vitro conditions and in vivo. In vitro-derived Th17 cells displayed high levels of IL-17 and IL-22, while Th17 cells isolated from S. aureus-infected mice exhibited a IL-10-producing phenotype. These results suggest that Th17 cells have distinct differentiation requirements and functional properties depending on the antigenic specificity and the priming conditions. In vivo, Th17 cells in the skin are initially pro-inflammatory, but subsequently acquire regulatory properties upon interaction with IL-10-producing cells, thereby providing a mechanism to control inflammation and prevent tissue damage. Our findings highlight the importance of IL-10 in the regulation of Th17 cell function and suggest that IL-10 may play a critical role in the development and maintenance of regulatory Th17 cells in the skin.
Selective toll-like receptor (TLR) stimulation reduces skin inflammation in a psoriasis mouse model
The authors investigated the effects of selective TLR stimulation on skin inflammation in a psoriasis mouse model. They observed that selective TLR stimulation reduced skin inflammation and improved the clinical symptoms of psoriasis. These findings suggest that selective TLR stimulation could be a potential therapeutic strategy for psoriasis.

The role of JAK-STAT signaling in the pathogenesis of psoriasis
In this study, the authors investigated the role of JAK-STAT signaling in the pathogenesis of psoriasis. They found that inhibition of JAK-STAT signaling reduced skin inflammation and improved the clinical symptoms of psoriasis. These findings suggest that inhibitors of JAK-STAT signaling could be a potential therapeutic strategy for psoriasis.

In situ induction of regulatory T cells in the skin of patients with atopic dermatitis
The authors investigated the in situ induction of regulatory T cells (Tregs) in the skin of patients with atopic dermatitis. They found that Tregs could be induced in the skin of patients with atopic dermatitis, and that these Tregs were capable of suppressing skin inflammation. These findings suggest that Treg induction in the skin of patients with atopic dermatitis could be a potential therapeutic strategy for this disease.

The role of the IL-17A pathway in the pathogenesis of atopic dermatitis
This study investigated the role of the IL-17A pathway in the pathogenesis of atopic dermatitis. The authors found that inhibition of the IL-17A pathway reduced skin inflammation and improved the clinical symptoms of atopic dermatitis. These findings suggest that inhibitors of the IL-17A pathway could be a potential therapeutic strategy for atopic dermatitis.

In situ activation of human skin resident T regulatory cells by autologous Langerhans cells
In this study, the authors investigated the in situ activation of human skin resident T regulatory cells (Tregs) by autologous Langerhans cells. They found that Tregs could be activated by autologous Langerhans cells in the skin, and that this activation could suppress skin inflammation. These findings suggest that the use of autologous Langerhans cells to activate Tregs in the skin could be a potential therapeutic strategy for skin inflammation.

The role of the IL-17A pathway in the pathogenesis of eczema
This study investigated the role of the IL-17A pathway in the pathogenesis of eczema. The authors found that inhibition of the IL-17A pathway reduced skin inflammation and improved the clinical symptoms of eczema. These findings suggest that inhibitors of the IL-17A pathway could be a potential therapeutic strategy for eczema.

The role of T helper 2 (Th2) cells in the pathogenesis of eczema
In this study, the authors investigated the role of T helper 2 (Th2) cells in the pathogenesis of eczema. They found that Th2 cells were involved in the pathogenesis of eczema, and that inhibition of Th2 cells reduced skin inflammation and improved the clinical symptoms of eczema. These findings suggest that inhibitors of Th2 cells could be a potential therapeutic strategy for eczema.

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019 The dual (activating/suppressive) effect of extracellular HIV-1 Tat protein is driven by the T cell polarizing capacity of myeloid dendritic cells in atopic dermatitis

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Background HIV-1 Tat protein, strongly associated with pathogenesis and/no antiviral therapy resistance, induces either an activation with enhanced production of IL2 or an immunosuppression of CD4+ T cells activation and viral replication) and at late stages (by inducing immunosuppression, inclusion, together with extracellular Tat and circulating antiviral IFN-gamma, inflammatory innate factors such as ATP and derivatives released by CPE-dead cultured cells. We show that, according to its concentration and the level of viral infection, extracellular Tat factors, as well as ATP and ATP-derivatives, Tat protein may exert either an activation with enhanced production of IL2 or an immune suppression of stimulated CD4+ T cells populations. The double-edged sword of Tat activity on CD4+ T cells challenges our understanding of inflammatory factors and the Treg-mediated blockage was abrogated by adding the A2A agonist 4aminopyridine, leading to decreased T cell adhesion. As Treg generate adenosine by cleavage of ATP via CD39, we provide an effective therapeutic strategy for the treatment of hyperkeratosis and parakeratosis triggered by a topical application of a TLR7/8 agonist, imiquimod. Langerhans cell-depletion by utilizing diphtheria toxin (DT) in Langerin-DT receptor-knocked-in-mice suppressed hyperkeratosis, parakeratosis, and neutrophil infiltration in the imiquimod-treated regions. In addition, depletion of the keratinocyte cell decreased the enhanced expression of STAT3 and Ki-67 in the epidermis induced by imiquimod. Our data show that Langerhans cells are required for generation of psoriasis-like lesions by imiquimod in mouse models.

021 The dual (activating/suppressive) effect of extracellular HIV-1 Tat protein is driven by the immune microenvironment of infected lymphoid DCs

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Background HIV-1 Tat protein induces the differentiation of naive CD4+ T cells into Th1 and Th2 cells, leading to decreased T cell adhesion. As Treg generate adenosine by cleavage of ATP via CD39, we provide an effective therapeutic strategy for the treatment of hyperkeratosis and parakeratosis triggered by a topical application of a TLR7/8 agonist, imiquimod. Langerhans cell-depletion by utilizing diphtheria toxin (DT) in Langerin-DT receptor-knocked-in-mice suppressed hyperkeratosis, parakeratosis, and neutrophil infiltration in the imiquimod-treated regions. In addition, depletion of the keratinocyte cell decreased the enhanced expression of STAT3 and Ki-67 in the epidermis induced by imiquimod. Our data show that Langerhans cells are required for generation of psoriasis-like lesions by imiquimod in mouse models.

020 Characterization of T-cell subsets and cytokine profiles in rejected skin grafts in an Epidermolysis Bullosa model system

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Patients suffering from skin disorders often have null mutations in genes encoding structural proteins of the skin. In junctional Epidermolysis bullosa (JEB), patients lack type XVII collagen (BP230) in the dermo-epidermal basement membrane zone (BMZ). Replacement defective genes in the skin by ex vivo gene therapy and subsequent transplantation of skin grafts is among the most promising approaches for the treatment of Epidermolysis bullosa. This cutaneous gene therapy is complicated by the risk of an immune response against the neo-antigen expressed in the skin graft. One critical aspect for the success of this treatment is the induction and maintenance of tolerance towards this neo-antigen. As a model system for this anti-graft response we use full-thickness skin from a transgenic mouse strain expressing BP230 and graft it onto syngeneic wild-type C57BL6 recipients. We previously demonstrated tolerance induction towards BP230 in vivo by delivery of its immune-oderm domain, NC1A, via gene gun transfection. Graft acceptance was achieved in 80%. Therefore the aim of the current study is the characterization of the immune response towards the antigen in in vitro co-culture. This model is able to selectively block rejection of BP230 expressing cells. Previous studies assume regulatory T-cells as master regulators of induced tolerance as in vivo depletion of Tregs leads to rejection of transplanted as well as already accepted grafts. This model may be used for evaluating strategies for blocking immune responses in pre-treated, untreated and Treg-depleted mice. Furthermore the mode of action of gene gun technology in mice skin should be ascertained in further detail.
Expression of IL-1 family members in positive patch test reactions and upon hapten challenge in uninvolved skin of allergic contact dermatitis patients

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The expression of IL-1 family members was assessed in healthy skin and in skin exposed to positive patch test reactions. The expression of IL-1 family members was higher in skin exposed to positive patch test reactions in comparison with healthy skin.

Tissue biomarkers in melanoma patients treated with TIL

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While treating stage III melanoma patients with autologous therapeutic TIL, we previously reported that both progression-free survival and overall survival were significantly increased in the group of patients with only one invaded lymph node. In this context, the main objective of the present study was to determine the difference in the characteristics of TIL expansions mediated to the patients between the two groups, and thus to determine if they could be predictive markers of response to adaptive immunotherapy using TIL. We confirmed that both PFS and OS were significantly associated to the presence of tumor-reactive T-cells among TIL injected to the patients. Moreover, our results identified that 3 markers involved in interactions between melanoma cells and T cells have a significant difference of expression between the 2 groups. MHC class I, adhesion molecule ICAM-1 and the co-stimulation molecule LFA-3. The expression of ICAM-1 was significantly correlated with OS.

Abrogation of human Langerhans' cell migration by inhibition of β1 integrin function

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Epidermal Langerhans' cells (LCs) are the outermost sentinel of the immune system, charged with maintaining immunological tolerance and translating novel antigenic stimuli into an appropriate immune response. Following antigenic stimulation, LCs detach from surrounding keratinocytes and migrate to local lymph nodes. Integrins are bidirectional signalling molecules involved in many facets of cellular physiology, migration and survival. We exist as heterodimers, with α and β subunits. Functional α and α4 integrins with presumed β1 integrin partners subunits are required for LC migration in mice. We have explored the role of β1 integrin in human LC migration using an epithelial explant assay. Four different mouse procollagen α1(1)I subunits were purchased from mice using α1(1)II collagen. In contrast, the β1 integrin subunit was significantly induced in the presence of tumor-reactive T-cells and melanoma patients with several invaded LN. In addition, the expression of the alpha chain of the IL-2 receptor (CD25) and the nuclear transcription factor Foxp3 was significantly increased in LN tissue specimens from the group of patients with smal screened LN disease stage. These results indicate that the host tumor microenvironment may emerge as a general predictor of possible clinical benefit in particular for treatment using TIL infusion.

A semi-automatic computational method to identify FOXP3+ T cell subpopulations in melanoma patients

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Accumulating evidence indicates that FOXP3+ CD4+ T cells do not comprise a homogeneous regulatory population, but are composed of different subpopulations. We have recently shown that both regulatory and non-regulatory FOXP3+ T cells are increased in melanoma patients, and that regulatory T cells from these patients have an aberrant phenotype. As is the case in almost all studies in immunology, in the previous study, we used a semi-automatic method to identify FOXP3+ T cell subpopulations in melanoma patients. In the current study, we employed a manual gating approach by professional immunologists to identify FOXP3+ T cell subpopulations. It is important to note that this method is limited in its ability to identify different populations simultaneously, and therefore to be used as a useful laboratory test for evaluating the immunological status of melanoma patients.

Therapeutic blockade of MALT1's protease activity as a tool to block mature T cell proliferation in the skin

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We have developed a new therapeutic tool that can block mature T cell proliferation in vitro and in vivo (in mice). We based our strategy on the recent identification of the protease activity of MALT1, a protein that links the NF-kB signal transduction and the actin cytoskeleton. Future experiments are planned to test the therapeutic effect of MALT1 inhibition in vivo.

Effects and mechanisms of citalopram treatment on marine contact allergic skin

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The purpose of this study was to determine the effects and mechanisms of citalopram (a selective serotonin reuptake inhibitor) treatment on marine contact allergic dermatitis (ACD). ACD patients were treated with citalopram (1 mg/kg) and the marine allergen. The results showed that citalopram inhibited the development of ACD in mice.

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Th17 cells in nummular dermatitis: an immunohistological and clinical study

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031
Alitretinoin – molecular and cellular mechanisms of action

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Chronic hand eczema (CHE) represents an inflammatory skin disease with a high prevalence ranging from 7-12% in Western industrialized countries. It was only starting from 2008 that the first, and until now the only, systemic treatment option, i.e. oral alitretinoin was approved in several countries for severe CHE unresponsive to potent topical corticosteroids. However, as the precise mechanism of actions (MOA) of alitretinoin in CHE are so far unknown, we undertook following investigations in order to shed some light on potential underlying immunomodulatory mechanisms. To investigate the mode of action of alitretinoin we stimulated human primary keratinocytes as well as leukocyte subsets and performed quantitative real-time PCR analyses, FACS-analyses, cell proliferation assays as well as mixed leukocyte reactions (MLR). Alitretinoin acts on keratinocytes as well as dendritic cells. Keratinocytes show a significant reduction of chemokine expression after stimulation with alitretinoin whereas on dendritic cells alitretinoin inhibits the upregulation of the maturation marker CD86 as well as the co-stimulatory molecules CD80 and CD86. Consequently, alitretinoin-treated dendritic cells show significantly impaired T cell activating properties. Further analyses demonstrated that the immunomodulatory effect of alitretinoin results in a marked suppression of allergenic proliferation of leukocytes in MLR. Taken together, these results suggest that alitretinoin modulates innate as well as adaptive immune responses by suppression of chemokine-induced leukocyte recruitment and inhibition of dendritic cell-mediated T cell activation.

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Evaluation of the correlation between regulatory T and plasmacytoid dendritic cells with grading of squamous cell carcinoma

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Regulatory T (Treg) and plasmacytoid dendritic cells (pDC) play a role in tumour immunity response. Indeed, Treg are involved in the process of self-tolerance, but they simultaneously might permit the development of neoplasms. On the other hand, pDC are implicated in both acquired and innate immunity, specifically against viral infections. However, the relationship between T reg, pDC populations in the presence of viruses and patient prognosis remains controversial. The current study aims to establish a correlation between inflammatory infiltrate markers and cutaneous squamous cell carcinoma (SCC) grading and determine the predictive value of Treg and pDC cell density in a cohort of SCC by using an immunohistochimical assessment. Forty SCC patients, twenty G1 (14 males and 6 females, mean age 79 years) and twenty G3 (12 males and 8 females, mean age 83 years) referring to the local Dermatology Unit, have been recruited and subjected to double immunohistochemistry for CD3/FOXP3 (Treg cells) and single staining for CD123 (pDC) and also for CD4 and CD8. The total number of FOXP3/CD25 Treg cells significantly correlated with the grading of the neoplasia and higher tumour grade. Since many studies indicate the involvement of β-HPV in skin carcinogenesis, the presence of these viruses will be also evaluated and correlated with pDC expression as these cells are well known producers of antiviral type 1 IFN and modulators of T cell response.

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