The IL-33-ST2 pathway drives mast cell-dependent antiviral responses

Recent studies have highlighted the new role of IL-33 as an "alarmin" in host defense. IL-33 has shown protective effects against infection with some pathogens in various organs such as the gut and peripheral cavity, however, it has not been reported in the skin. We have previously reported that mast cells were critically involved in host defense at herpes simplex virus 2 (HSV-2)-infected sites through TNF-α and IL-6 production using a murine model of HSV encephalitis. We also found that IL-33 released from HSV-infected epidermis activates mast cells to secrete these proinflammatory cytokines. In addition, IL-33 receptor ST2+ mice exhibited increased clinical severity and mortality following cutaneous HSV-2 infection, indicating that the IL-33 contributes to protective antiviral responses. Taken together, our findings suggest the involvement of the IL-33-ST2 pathway in mast cell function in the context of antiviral innate immunity.

Neutrophil extracellular trap-derived cathelicidin antimicrobial peptide: contribution to macrophage-dependent resistance

We are currently testing whether cathelicidin as part of whole NETs and DNA-cathelicidin complexes show activity against intracellular pathogens. To study cathelicidin as part of whole NETs we isolated neutrophils, trap pathogens. A characteristic feature of NETs is the expression of cathelicidin and other antimicrobial peptides. Accordingly, NETs are a source of self-DNA-antimicrobial peptide complexes. Phagocytes take up whole NETs, as well as DNA-antimicrobial peptide complexes. However, whether NETs and DNA-antimicrobial peptide complexes contribute to the cooperative antimicrobial responses by human neutrophils and macrophages against intracellular pathogens is not clear. Here, we investigate whether cathelicidin as part of NETs and DNA-cathelicidin complexes contribute to the cooperative antimicrobial activity against intracellular pathogens. To study cathelicidin as part of whole NETs we isolated NETs from activated primary human neutrophils. To study cathelicidin in the context of DNA-antimicrobial peptide complexes we generated DNA-cathelicidin complexes by incubating cathelicidin with DNA complexes by incubating cathelicidin with DNA. Neutrophils were then infected with bacteria and cathelicidin activity was measured in a lysozyme lysis assay as well as by a C-terminal cationic fragment of filaggrin-2 (FLG24), a structural protein of human skin, has been found to be highly active against preferentially soil- and waterborne bacteria such as several Pseudomonas species and Escherichia coli. Transmission electron and confocal laser scanning microscopy analyses showed that FLG24 was not only able to induce bleb formation in P. aeruginosa, but also could be localized on or in those blebs. Both a lysozyme lysis assay as well as the NETosis assay did not reveal any pore formation in the bacterial membrane by FLG24. Strikingly, the latter indicated a competition in DNA-binding between the tested antimicrobial protein and the DNA-binding dye. A preferential binding of FLG24 to DNA rather than to P. aeruginosa DNA. A role of the DNA-binding domain is essential for bacterial replication, suggesting that FLG24 can impede the bacterial replication process. In a simplified model for replication, FLG24 showed inhibiting effects on PCR reactions. The higher the FLG24 concentrations in the reactions, the weaker were the corresponding band intensities of PCR amplicons. Comparable amounts of hHBD or BSA did not show any effects. Additionally, in an in vivo plasmid replication assay using chloramphenicol-treated E. coli harboring the plasmid pBluescript, FLG24 was able to inhibit pBluescript replication in the translationally inactive E. coli compared to untreated bacteria. Taken together these results indicate that the antibacterial activity of FLG24 against P. aeruginosa and E. coli is based on the impairment of DNA replication, thereby inhibiting cell division and finally causing bacterial death.

A link between netting neutrophils and plasmacytoid dendritic cells in skin wound healing

Netting neutrophils control infiltration and activation of pDCs in the injured skin via CXCL10. The mechanisms that regulate healing of the injured skin are not well understood. We have previously shown that plasmacytid dendritic cells (pDCs) are normally absent from the healthy skin, but rapidly invade both mouse and human skin upon injury. Upon skin infection, pDCs sense nucleic acids via TLR7/TLR9 and are activated to produce type I IFN, a process that is crucial for reepithelialization of skin wounds. However, the mechanisms that drive pDC recruitment and activation in injured skin remain unclear. We found that neutrophils, the first cells recruited to the injured skin, were directly responsible for pDC recruitment as neutrophil depletion abrogated pDC infiltration. In addition, we observed that infiltrating neutrophils release Neutrophil Extracellular Traps (NETs) composed of DNA filaments decorated with granule-derived proteins. Strikingly, blocking Netosis inhibited pDC recruitment and activation, and injection of netting neutrophils to the dermis was sufficient for pDC infiltration and activation. Importantly, we found that neutrophil-driven pDC recruitment and activation was dependent on CXCL10, constitutively expressed in neutrophil granules and released in the context of NETosis. Interestingly, CXCL10 was found to form complexes with DNA and to activate pDCs via TLR9, in addition to their chemotactic activity. Accordingly, when injected into skin, CXCL10 led to both pDCs infiltration and activation. Altogether, these data demonstrate that netting neutrophils control infiltration and activation of pDCs in the injured skin via CXCL10. Our findings provide new insights into the mechanisms of wound healing and open new avenues for potential therapeutic interventions to boost or inhibit wound repair in the skin.

Post-septic immune-suppression following Gram positive sepsis is mediated by TLR dependent induction of myeloid derived suppressor cells (MDSCs)

MDSCs are a subpopulation of monocytic cells present in various tissues and peripheral blood in pathogen-activated states. MDSCs are capable of inhibiting T-cell responses against tumors or viral infections and also have been implicated in the development of immune tolerance in autoimmune diseases. MDSCs have been shown to be induced in the peripheral blood and tissues of patients with sepsis. However, the mechanisms underlying the induction of MDSCs in sepsis are not fully understood. We hypothesized that TLR signaling is involved in the induction of MDSCs in sepsis. To test this hypothesis, we used a mouse model of Gram positive sepsis induced by intraperitoneal injection of Staphylococcus aureus. We found that TLR signaling is involved in the induction of MDSCs in sepsis. MDSCs were significantly increased in the peripheral blood and spleen of septic mice compared to control mice. Furthermore, we found that TLR signaling is necessary for the induction of MDSCs in sepsis. In TLR knock-out mice, MDSCs were not induced in response to sepsis. These findings suggest that TLR signaling is involved in the induction of MDSCs in sepsis. Furthermore, our findings have implications for the development of novel therapies to target MDSCs in sepsis.
436 MiR-146a is a potent regulator of TLR2- and IL-1β-induced inflammatory responses in keratinocytes

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Keratinocytes represent the first line of defense against pathogens in skin; they recognize patho-
gens by pattern-recognition receptors such as Toll-like receptors (TLRs) and initiate an inflamma-
tory response. Although much is known about the innate immune functions of keratinocytes, the
mechanisms that prevent excessive inflammation are not well understood. MicroRNAs are short,
endogenous RNAs that regulate gene expression. Here, we investigated the role of miR-146a during
the innate immune response of keratinocytes. Stimulation of primary human keratinocytes with
Toll-like receptor 2 (TLR2) ligands or IL-1β resulted in a NF-κB and MAPK-dependent induction of
miR-146a expression. Surprisingly, a single stimulation with TLR2 ligands or IL-1β resulted in a
long-lasting up-regulation of miR-146a, contrasting the rapid and transient expression of inflamma-
tory mediators (e.g. IL-8, CCL20, TNF-α). Overexpression of miR-146a suppressed the production of
IL-8, CCL20 and TNF-α, which functionally suppressed the chemotactic attraction of neutrophils
by keratinocytes. Anti-miR-146a increased endogenous miR-146a induced the production of inflammatory mediators even in resting, non-stimulated keratinocytes, and potentiates the effect of TLR2-stimu-
lation. Transcriptomic profiling revealed that miR-146a suppresses the expression of a large number of
proinflammatory-like genes in keratinocytes, including epidermal growth factors, and aberrant keratino-
tocyte differentiation one week after skin injury. The development of a psoriatic phenotype was
critically dependent on type 1 IFN as blockade of IFN signaling completely abrogated the effect of
TNF inhibition. These data suggest the Yin-yang model, in which TNF controls IFN-alpha expression
by pDCs, and that a crosstalk between skin inflammation results from persistent unbalanced IFN-alpha production by pDCs, completely independent of underlying disease.

437 Effects of S. aureus and S. epidermidis from atopic children microbiota on TCD4+ cell sub-sets

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Atopic dermatitis (AD) has been recently revisited with respect to composition of skin microbiota. Interactions between the immune system and pathogenic or commensal skin bacteria is of major interest to the pathophysiology of AD. To assess the role of skin microbiota in TCD4+ polarization, we investigated a cohort of children with AD (n=17) vs non-AD (n=22). We also compared AD to the microbiota of healthy controls. Our study shows a prevalence of IL-4+ and IFN-γ-producing T cells in AD, a Th2 feature confirmed by qRT-PCR on infected skin samples by upregulation of GATA3, IL-13, CCL22 and CCL17. To understand how AD skin microbiota (S. aureus and S. epidermidis) could influence TCD4 cell polarization, monocyte-derived dendritic cells (MoDC) from blood donors were stimulated with bacteria secretion products prepared from AD and non-AD. SA-conditioned MoDC secreted only IFN-γ while SE-MoDC produced only IL-10. IL-17 was expressed at a higher level in AD, which could block IFN-γ-induced Th1 cell differentiation of Th17. Overall, our data suggest that S. aureus products, including superantigen (SAg), induced IFN-γ secretion by MoDC, increasing IL-17 expression in a Th2-prone microenvironment. These preliminary results raise several questions regarding the role of TCD4+ cell subsets in the pathogenesis of AD.

438 Anti-TNF promotes type I interferon-driven psoriasis-like skin inflammation

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Acute flares of psoriasis are a well-known side-effect of anti-TNF therapy, although the pathogenic mechanism has remained elusive. We analyzed a series of 33 cases of pre-existing psoriasis patients on anti-TNF agents. While only 10% showed a flare of psoriasis, 18% of the patients developed a psoriasis-like skin lesion. Overexpression of miR-146a suppressed the production of IL-8, CCL20 and TNF-α, which functionally suppressed the chemotactic attraction of neutrophils by keratinocytes. Anti-miR-146a increased endogenous miR-146a induced the production of inflammatory mediators even in resting, non-stimulated keratinocytes, and potentiated the effect of TLR2-stimulation. Transcriptomic profiling revealed that miR-146a suppresses the expression of a large number of proinflammatory-like genes in keratinocytes, including epidermal growth factors, and aberrant keratino-
tocyte differentiation one week after skin injury. The development of a psoriatic phenotype was
critically dependent on type 1 IFN as blockade of IFN signaling completely abrogated the effect of
TNF inhibition. These data suggest the Yin-yang model, in which TNF controls IFN-alpha expression
by pDCs, and that a crosstalk between skin inflammation results from persistent unbalanced IFN-alpha production by pDCs, completely independent of underlying disease.

439 Role of neutrophils in the pathogenesis of imiquimod-induced psoriasis-like skin lesions

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Psoriasis is a common cutaneous disorder characterized by chronic inflammation and erythema-
tous plaques. The thickening of the skin has been denoted by enhanced epidermal proliferation, which is a hallmark of psoriasis pathogenesis. While T cells are recognized as central to the key patho-
 genetic inflammatory circuits. Their in vitro effects have been tested in normal human keratinocytes, but their role in the pathogenic role of psoriasis-signature cytokines in the development and progression of psoriasis-like skin lesions. Here, we performed a comprehensive gene expression analysis investigating the overall alterations in human adipose tissue in the pathogenesis of psoriasis as source of pro-inflammatory mediators. We will also test few cytokines in the development and progression of adipocyte-related disorders. We firstly tested few key inflammatory genes, namely CCL20, IL-23, IL-17, TNF-α, IFN-γ, chemokines (CCL20), IL-10, and other pro-inflammatory mediators, which may participate to psoriasis skin inflammation and to the pathogenesis of psoriatic comorbidities. Among psoriasis-signature cytokines, IL-17 and TNF-α are recognized as central to the key patho-
genic inflammatory circuits. Their in vitro effects have been tested in normal human keratinocytes, but no data are available regarding their combined effect on normal adipose tissue. Thus, we examined their effect on human keratinocytes cultured with or without neutrophils. Our findings provide insight into the interaction between immune system and dermal fibroblasts in inflammatory skin diseases.

440 Activation of Toll-like receptors alters microRNA expression in keratinocytes

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Keratinocytes in the skin represent the first line of defense against pathogens. They recognize micro-
organisms by pattern-recognition receptors, among others Toll-like receptors (TLRs), and initiate a cascade of signaling events that lead to an inflammatory response. The role of miRNAs in this process is as yet unexplored. Here, we aimed to identify miRNAs that are regulated in keratinocytes upon treatment with different TLR ligands. MicroRNA expression profiling of keratinocytes treated with zymosan (a ligand of TLR2), flagellin (a ligand of TLR5) and the viral RNA-antagonist poly(I:C) (a ligand of TLR3), identified specific sets of miRNAs significantly regulated by these TLR ligands. MiRNAs were regulated in a concentration-dependent and TLR ligand-specific manner. One miRNA was outstanding regarding its expression as it was strongly induced by all treatments in most time points: miR-146a. Our results provide a basis for further studies to unveil the role of miRNAs in keratinocytes and may lead to a better understanding of skin innate immune responses, which are often altered in inflammatory skin diseases.

441 Adipose tissue response to IL-17 combined with TNF-α exposure

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Psoriasis is a chronic inflammatory skin disease characterized by activation of antigen presenting cells and T cells, inducing a tissue response. As occurs with other immune-mediated inflammatory diseases, psoriasis is associated with a higher risk of developing “systemic” comorbidities including cardiovascular diseases, dismetabolic disorders, hypertension, and depression. Pathogenically, psoriasis is characterized by complex pathogenic mechanisms, involving multiple cytokines (i.e., IL-23, IL-17, TNF-α, IFN-γ), chemokines (CCL20), mitogens, and other pro-inflammatory mediators, which may participate to psoriasis skin inflammation and to the pathogenesis of psoriatic comorbidities. Among psoriasis-signature cytokines, IL-17 and TNF-α are recognized as central to the key patho-
genic inflammatory circuits. Their in vitro effects have been tested in normal human keratinocytes, but no data are available regarding their combined effect on normal adipose tissue. Thus, we examined their effect on human keratinocytes cultured with or without neutrophils. Our findings provide insight into the interaction between immune system and dermal fibroblasts in inflammatory skin diseases.

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Immunology 2: Innate Immunity and Microbiology | ABSTRACTS
**Demodex folliculorum** among patients with different clinical stage of Rosacea – a preliminary report of novel diagnostic procedure

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Introduction & Objectives: Rosacea is a chronic disease characterized by (depending on the subtype) facial erythema, telangiectasia, sometimes papules or nodules. Rosacea affects all ages and sex with four subtypes. Pathophysiology aims to many different trigger factors like sun exposure, emotional stress or changed intestinal flora. Studies about Demodex mites among different authors revealed that they may play a role in rosacea exacerbation in particular along with other triggers. Material & Methods: The study included patients with rosacea from 25 to 60 years of age. The patient status and content of hair follicles were assessed during two visits in our outpatient clinic. During the first visit, detailed medical history was taken, physical examination and hypoalgesic adhesive were applied. Results: Initially, significant from hair follicles of twenty patients with rosacea have been examined. Based on the results from our study there were no prominent increase in number of mites among our patients comparing to general population. There was a varying number of different phases of life cycle (ovum, larva, nymph, adult) in different patients. The results of our analysis will be presented in the final version of this paper. Conclusion: Based on the preliminary results, we can state that among our patients Demodex mites were not a main trigger factor. We have frozen collected mites for further analysis.

**Death and survival factors are co-expressed on T cells in SLE patients**

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Dendritic cells (DCs) are of particular significance in the pathogenesis of human T-cell leukemia virus type 1 (HTLV-1) infection. HTLV-1 infected T cells (HTLV-1+ T cells) are highly infectious for other T cells when translocated to other sites, they may become highly infectious, for example in brain and lung disc infections. In order to further investigate the difference of the genotypes, we analysed the genome differences were considerable by whole genome comparisons (p < 0.01). In this study, we showed that CD-T cells VS formation contributes to HTLV-1 infection as well as functional suppression on DCs.
Identification of the signaling pathway mediating the anti-inflammatory effect of cannabidiol on human sebocytes

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We have previously shown that endocannabinoids (e.g., anandamide, 2-AG) act as negative regulators and anti-inflammatory actors. We have also demonstrated that the lipidic and growth-inhibitory effects of CBD are mediated by the activation of transient receptor potential vanilloid 4 (TRPV4) channels; however, the molecular mechanism of its anti-inflammatory action is still unknown. Since the anti-inflammatory effect of CBD in a mouse model of dermatitis is associated with decreased expression of IL-17A and TNF-α, we aimed at determining whether CBD activates the endocannabinoid system (ECS) and which endocannabinoids are involved in this process. To this end, we cultured human SZ95 sebocytes with CBD and with a selective TRPV4 activator (AM-404) and determined their expression profile of the ECS. In addition, we performed reverse transcription real-time PCR (qPCR) to determine the expression profile of selected pro-inflammatory genes.

Our results suggest that CBD activates the ECS and that 2-AG and anandamide are involved in this process. These findings are in line with other studies showing a link between ECS activation and anti-inflammatory effects of CBD in vitro. Furthermore, these findings have potential clinical implications. Sebocytes play important roles in the maintenance of the basal lipid synthesis of the sebocytes. In contrast, (-)-cannabidiol (CBD), the major non-psychoactive phytocannabinoid of Cannabis sativa, was found to suppress unsterilized sebaceous lipogenesis and exert anti-inflammatory and anti-atherogenic activities. These findings suggest that CBD may exert an anti-inflammatory effect on sebocytes and reduce sebaceous lipogenesis and sebum production. Further studies are required to elucidate the molecular mechanisms underlying the anti-inflammatory effects of CBD on sebocytes.

Withdrawn

Propionibacterium acne (P. acne) and sebaceous glands are considered to play an important role in the development of acne. However, information regarding the activation of innate immunity by P. acne in the sebaceous gland is limited. In this study, we investigated whether P. acne activates the inflammasome in human sebaceous glands in vivo and in vitro. We found that IL-1β expression was upregulated in sebaceous glands of acne lesions. After stimulation of human SZ95 sebocytes with P. acne, the activation of caspase-1 and secretion of IL-1β were enhanced significantly. Moreover, knocking down the expression of NLRP3 (but not AIM2) abolished P. acne-induced IL-1β production in SZ95 sebocytes. The activation of the NLRP3 inflammasome by P. acne was dependent on protease activity and ROS generation. Finally, we found that NALP1-deficient mice displayed reduced inflammatory responses to P. acne. Treatment of human sebocytes with P. acne-induced IL-1β activation in sebaceous glands plays an important role in acne pathogenesis.

Propionibacterium acne (P. acne) bacterium is a member of the skin microflora, but may also serve as an opportunistic pathogen contributing to the pathogenesis of different skin diseases. Earlier we have shown that various P. acne strains (809, 6609, ATCC 11826) belonging to different phylogroups within the species differentially affected the proliferation and viability of cultured HPV-KER cells, and that this strain specificity, the extent of the induced cell biological changes greatly depended on the dose of the bacterial treatment; however, it is not well understood which bacterial factors contribute to the pathogenesis in vivo. To study this concept we investigated the humoral and cellular immune response to various P. acne strains in the context of IL-1β production.

The results show that P. acne strains, belonging to different phylogroups differentially affect the proliferation and viability of cultured HPV-KER cells. Moreover, the amount of free hemoglobin increased. The same strain specificity was observed in the in vitro assays, where bacterial treatment was applied to HPV-KER cells at different doses. However, the amount of free hemoglobin increased only in the case of the strains belonging to different phylogroups, while the same strain specificity was observed in the in vitro assays, where bacterial treatment was applied to HPV-KER cells at different doses. However, the amount of free hemoglobin increased only in the case of the strains belonging to different phylogroups. These findings suggest that strain specificity is crucial for the pathogenesis of acne vulgaris. Further studies are required to elucidate the molecular mechanisms underlying the strain specificity of P. acne in the pathogenesis of acne vulgaris.

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen notorious for its ability to resist the host's immune response. This species is known for its immune evasive abilities amongst others by degradation of a large variety of host proteins. However, it has not been confirmed whether P. aeruginosa degrades host proteins in vivo. The aim of this study was to investigate whether P. aeruginosa can degrade host proteins that are involved in host defense. To study this concept we investigated human defensins and human beta-defensins as model proteins. We used a broad range of methods to detect the presence of these proteins in the culture supernatants of P. aeruginosa cultures. Our results show that P. aeruginosa degrades host proteins in vivo. However, it is not clear whether these proteins are degraded by P. aeruginosa or by other bacteria. Further studies are required to elucidate the molecular mechanisms underlying the degradation of host proteins by P. aeruginosa.

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen notorious for its immune evasive abilities amongst others by degradation of a large variety of host proteins. However, it has not been confirmed whether P. aeruginosa degrades host proteins in vivo. The aim of this study was to investigate whether P. aeruginosa can degrade host proteins that are involved in host defense. To study this concept we investigated human defensins and human beta-defensins as model proteins. We used a broad range of methods to detect the presence of these proteins in the culture supernatants of P. aeruginosa cultures. Our results show that P. aeruginosa degrades host proteins in vivo. However, it is not clear whether these proteins are degraded by P. aeruginosa or by other bacteria. Further studies are required to elucidate the molecular mechanisms underlying the degradation of host proteins by P. aeruginosa.
454

Reduced pro-inflammatory activity and a delayed barrier repair in beta-defensin 14 deficient mice

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Mouse beta-defensin 14 (mBD-14), the orthologue of human beta-defensin 3, has a broad antimicrobial activity and exhibits chemotactic activity for T cells. Inflammatory signals including cytokines are important for skin barrier repair. We asked whether a deficiency in mBD-14 expression leads to a delay in permeability barrier repair and asked for the mechanisms. Skin barrier disruption in mBD-14 deficient mice is characterized by a delay in barrier repair after tape-stripping as compared to wild-type mice. Topical application of a solution of 1% mBD-14 partially reversed the delay in permeability barrier repair. The inflammatory cell infiltrate and the induction of IL-1β after barrier disruption were reduced in mBD-14 deficient compared to wild mice and normalized by topical application of mBD-14 protein. Also, the increase in proliferation and the increase in epithelial thickness induced by tape stripping in wild-type were reduced in mBD-14 deficient mice and enhanced by topical application of mBD-14. The increase in the expression of differentiation markers involucrin, and filaggrin induced by tape-stripping was also reduced in mBD-14 deficient and influenced by topical mBD-14. We suggest that the delay in permeability barrier repair in mBD-14 deficient mice may be related to the proinflammatory and chemotactic activity of this defensin. It was shown that mutated mBD-14 has a reduced antimicrobial activity which is important for skin barrier repair.

455

The negative regulatory factors of the Propionibacterium acneis-induced signaling pathways in vitro cultured immortalized keratinocytes

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Propionibacterium acneis (P. acneis) is a commensal bacterium, but it can also activate different pathogen recognition receptor (e.g. Toll-like receptor; TLR) and induce innate immune and inflammatory events in human epidermal keratinocytes. These molecular pathways are well characterized, but little is known about the endogenous negative regulatory mechanism that may control the release of pro-inflammatory cytokines. To investigate the negative regulatory signaling processes in response to P. acneis, we used experimental autoimmune encephalomyelitis (EAE) in mice, an animal model of multiple sclerosis, which is induced in C57BL/6j mice by injection of the myelin-oligodendrocyte-glycoprotein (MOG33-55). Mice were injected intra-novically with 20 μg mBD14 prior to immunisation with MOG33-55 and the severity of EAE was monitored. Treatment with mBD14 attenuated the clinical score significantly in comparison to positive controls which developed progressive paralysis upon MOG33-55 injection. Histopathology of central nervous tissue (CNS) confirmed the beneficial effect of mBD14 in the decrease of inflammatory infiltrate and preserved tissue integrity. The increased numbers of CD4+ and CD8+ T cells found in the lymph nodes of EAE mice were reduced upon injection of mBD14. The same applied for the release of interferon-γ and tumor necrosis factor-α by lymph node cells. FACs analysis of mononuclear cells isolated from the CNS revealed a significant upregulation of the Treg marker Foxp3 and GARP upon mBD14-treatment. These findings provide a clearer understanding of the mechanism of current drugs while also providing a framework to test and develop novel therapies inhibiting NETosis.

456

Sulfasalazine and thalidomide inhibit extracellular trap formation by human neutrophils

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Neutrophil extracellular trap formation (NETosis) is a recently discovered form of cell death distinct from necrosis or apoptosis where a lattice of DNA strands is extruded from innate immune cells. Although NETosis is typically thought of as an antimicrobial response, recent work in our lab has shown that mast cells and neutrophils form IL17+ extracellular traps (ET) in human psoriasis plaques but not in normal uninvolved skin. In autoinflammatory diseases, overproduction or insufficiency of ETs may lead to inappropriately sustained stimulation of the innate immune system. However, while several important treatments for psoriasis and other autoimmune diseases target TNF-α, IL17, and IL23, drugs that inhibit NETosis have not been identified. Several drugs used today to treat autoimmune diseases work through incompletely understood mechanisms of action. We hypothesized that the clinical effectiveness of some of these drugs may be attributed in part to their inhibition of NETosis. Using neutrophils isolated from human donors, we investigated the effects of various drugs on NETosis. Through immunofluorescence and assays measuring extruded ET DNA, we demonstrate that sulfasalazine, thalidomide, and to a lesser extent DMSO, inhibit NETosis of human neutrophils. In contrast, cyclosporine, tacrolimus, tetracycline, colchicine, hydroxychloroquine, cromolyn, or dapsone had no significant effect on NETosis in these experiments. The clinical effectiveness of sulfasalazine and thalidomide in treatment of diseases such as psoriasis, and pyoderma gangrenosum may therefore be attributable to blockade of NETosis. These findings provide a clearer understanding of the mechanism of current drugs while also providing a framework to test and develop novel therapies inhibiting NETosis.

457

The anti-peptide peptide murine beta defensin-14 suppresses progression of experimental autoimmune encephalomyelitis by the induction of regulatory T cells

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Beta-defensins are antimicrobial peptides (AMP) of the innate immune system produced by skin by various stimuli, including propionibacterial cytokines, bacterial infection and UV radiation (UV). Using the contact hypersensitivity model, we recently demonstrated that the UV-inducible murine AMP beta-defensin-14 (mBD14) can inhibit sensitization via the induction of regulatory T cells (Treg). To prove whether mBD14 exerts similar suppressive effects in other immunologic models, we used experimental autoimmune encephalomyelitis (EAE) in mice, an animal model of multiple sclerosis, which is induced in C57BL/6j mice by injection of the myelin-oligodendrocyte-glycoprotein (MOG33-55). Mice were injected intra-novically with 20 μg mBD14 prior to immunisation with MOG33-55 and the severity of EAE was monitored. Treatment with mBD14 attenuated the clinical score significantly in comparison to positive controls which developed progressive paralysis upon MOG33-55 injection. Histopathology of central nervous tissue (CNS) confirmed the beneficial effect of mBD14 in the decrease of inflammatory infiltrate and preserved tissue integrity. The increased numbers of CD4+ and CD8+ T cells found in the lymph nodes of EAE mice were reduced upon injection of mBD14. The same applied for the release of interferon-γ and tumor necrosis factor-α by lymph node cells. FACs analysis of mononuclear cells isolated from the CNS revealed a significant upregulation of the Treg marker Foxp3 and GARP upon mBD14-treatment. Together, these data indicate that mBD14 mitigates EAE presumably via the induction of Treg and confirm the previously described immunosuppressive features of AMP.
460 The thrombin-derived peptide GKY25 modulates endotoxin-induced responses through direct interactions with macrophages and monocytes
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Cationic host defence peptides have recently gained much interest as novel anti-infectives due to their ability to kill bacteria and simultaneously modulate bacterial induced host cell responses. C-terminal host defence peptides of human thrombin are found in human wounds and fibrin. Previous studies have shown that a prototypic peptide, GKY25, derived from the C-terminus of human thrombin, is anti-inflammatory in vitro and in vivo, but the mode of action is unclear. This study demonstrates that GKY25, apart from binding lipopolysaccharide (LPS), also directly interacts with monocytes and macrophages. The peptide reduced TLR4 and -2 induced NF-κB activation, and inhibited LPS-induced TLR4/MD2 dimerization. Cells pretreated with GKY25 showed diminished LPS-responses, and FACS analyses demonstrated that the peptide bound to monocytes and that this interaction was retained in human blood. Peptide binding to cells was abrogated at low temperature. GKY25 inhibited several microbe-derived agonists, including LPS, but also LTA, zymosan, and peptidoglycan. Taken together, the cell- and membrane-binding property of GKY25 mediates inhibition of TLR4-dimerization and subsequent reduction of NF-κB activity and pro-inflammatory cytokine production in monocytes and macrophages. Thus, host defence peptides of thrombin may be interesting therapeutic candidates for reduction of excessive inflammation and infection in various clinical settings.

461 The use of transgenic reporter mice and intravital multiphoton microscopy to study mast cell development in vivo
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Mast cells develop from a bone marrow-derived population of the myeloid lineage and are distributed strategically at environmental interfaces including the dermis of the skin whereby they are thought to play a critical role in the innate immune response to a range of pathogens in particular parasites. They are however also implicated in inflammatory and neoplastic conditions whereby mast cell (MC) numbers are increased in the skin. The mechanisms underlying this accumulation of mast cells is unclear and specifically, it is unknown to what extent local/reactive mastocytosis is mediated by in situ MC proliferation versus recruitment of MC progenitors. We have developed a novel imaging and flow cytometry-based platform that allows the characterization of the dynamics of MC proliferation within the skin at the single cell level in vivo. Using Kit-eGFP MC reporter mice, we have quantified the spatio-temporal proliferation of connective tissue MC repopulation in ear following inflammation. While skin mast cells are self-renewing and radiosensitive during steady-state, MC repopulation following inflammation was mediated by bone marrow-derived progenitors. Following their differentiation, these donor-derived mast cells proliferated within the skin and MC density was restored to that of wild type levels within 6 weeks. We propose a model of homing of inflammation-responsive MC progenitor recruitment that has implications for the pathogenesis of cutaneous mastocytosis.