362 Critical Role for Mast Cells in IL-1β-Driven Skin Inflammation Accompanying a Disease-Associated Micronutrient Abnormality

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Cryopyrin-associated periodic syndromes (CAPS) are caused by aberrant IL-1β production induced by NLRP3 mutations which can be recapitated in mice with disease-associated mutant Nlrp3 (Nlrp3-KI mice). Similar to CAPS patients, Nlrp3-KI mice exhibited delayed growth and reduced body weight accompanying with dermatitis rich in neumophils. However, the mechanism that orchestrates Nlrp3-dependent IL-1β-driven inflammatory disease remains unclear. We have uncovered a role for the indigenous microbiota and mast cells (MCs) in triggering IL-1β-mediated inflammation in skin of Nlrp3-KI mice. Co-segregation with IL-1β overexpressing mutant Nlrp3 induced IL-1β in response to LPS or Tnfα in vitro. MC deficiency greatly reduced disease in Nlrp3-KI mice and reconstitution of MC-deficient mice (Nlrp3-KI K674W/SHi/wi) with MC restored skin disease which is inhibited by anti-IgE, anti-CD11b, or anti-CD206 antibodies. The Nlrp3-KI mice reconstituted Nlrp3-KI K674W/SHi/wi mice suggesting that skin inflammation induces IL-1β production in blood leading to systemic changes in Nlrp3-KI mice. Surprisingly, neutralization of Tnfα or abrogated IL-1β production and skin disease in neonatal Nlrp3 mutant mice. In neonatal mice, the microbiota induced Tnfα and IL-1β from MCs and promoted skin disease. Intradermal Tnfα administration triggered inflammation in Nlrp3-KI mice which was inhibited in the absence of IL-1β or MC deficiency. Thus, the microbiota and MCs initiate cellular events leading to dysregulated IL-1β production and skin inflammation in mice with CAPS-associated Nlrp3 mutation.

364 Isolation of an antifungal protein from human skin

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Skin infections caused by fungi are affecting the population worldwide, in which T. rubrum is the most prevalent pathogen. Because of its rigid and immunosuppressive cell wall, T. rubrum is resistant to antifungal proteins (AMPs) recently isolated from human skin such as human β-defensins (HBDs) and the cathelicidin LL-37. We hypothesized the presence of specific antifungal proteins and isolated the T. rubrum-killing antifungal protein from human skin. As a source for isolation of skin-derived antifungal antimicrobial proteins, we collected the stratum corneum from psoriatic patients, atopic dermatitis patients, and healthy subjects. The protein extracts from each purification step were tested T. rubrum-killing activity in microbroth dilution antifungal assay. Since the protein extracts from psoriatic patients showed the most potent antifungal activity against T. rubrum, the fungus-specific antifungal protein from psoriatic stratum corneum was identified as a novel antifungal protein with potent activity against phytopathogenic fungi. In conclusion, we report here a protein with strong antifungal activity isolated from human skin with the most potent activity against phytopathogenic fungi in vitro. In this study, we report a protein with strong antifungal activity isolated from psoriatic stratum corneum, which probably plays a key role in preventing human skin from fungal infections.

365 Microbiome Dynamics of Human Epidermis Following Skin Barrier Disruption

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Recent advances in sequencing technologies have enabled metagenomic analyses of human bodies sites including gut, oral cavity, vagina and skin. Several studies have catalogued the composition of bacterial communities of the surface of human skin, mostly under static conditions in healthy volunteers. The response of the skin to environmental noxae is decisively shaped by the status of the immune system. In this setting, we have developed a protocol to analyze the skin microbiota of the surface layer and the deeper layers of the stratum corneum of normal skin, and investigated how cutaneous innate immune signals translate into different qualities of immune responses and clinical outcomes between resident bacteria and epidermis. To investigate how cutaneous innate immune signals translate into different qualities of immune responses and clinical outcomes, we established a skin inflammation model based on TLR2 signals and the contact sensitization model. We applied TLR2 ligands Pam3Cys-SK peptide or TLR2/1 ligand PamCys to FITC sensitized mice to mimic the presence of Gram positive bacteria. As read out of the TLR2/1 mediated inflammatory response, FITC contact hypersensitivity (CHS) at the site of challenge was investigated one week later. Surprisingly, cutaneous exposure to TLR2/6 ligands did not enhance but almost completely abrogated consistently elicited FITC specific CHS. In line with this, RT-PCR analysis showed a strong down-regulation of IL-4, IL-6 and IL-10 in these skin samples only. At the same time, CD1+ T cells and the number of activated IFNγ producing T cells significantly decreased in these skin samples. In parallel, we found a significant increase of G1c CD11b+ Gr1+CD11b+CD4+CD123+ cells, which represent macrophages. The results suggest that TLR2/6 ligands may dampen skin inflammation and initiate regulatory immune responses in the skin. In conclusion, we have developed a skin inflammation model based on TLR2 signals and the contact sensitization model. We applied TLR2 ligands Pam3Cys-SK peptide or TLR2/1 ligand PamCys to FITC sensitized mice to mimic the presence of Gram positive bacteria. As read out of the TLR2/1 mediated inflammatory response, FITC contact hypersensitivity (CHS) at the site of challenge was investigated one week later. Surprisingly, cutaneous exposure to TLR2/6 ligands did not enhance but almost completely abrogated consistently elicited FITC specific CHS. In line with this, RT-PCR analysis showed a strong down-regulation of IL-4, IL-6 and IL-10 in these skin samples only. At the same time, CD1+ T cells and the number of activated IFNγ producing T cells significantly decreased in these skin samples. In parallel, we found a significant increase of G1c CD11b+ Gr1+CD11b+CD4+CD123+ cells, which represent macrophages. The results suggest that TLR2/6 ligands may dampen skin inflammation and initiate regulatory immune responses in the skin.
638
A Novel Human Papillomavirus/Vaccine (RG1-VLP) with Efficacy against Mucosal and Cutaneous HPV

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Licensed human papillomaviruses (HPV) vaccines are based on virus-like particles (VLP), self-assembled from minor capsid protein L1. Both vaccines prevent persistent infection with mucosal high-risk HPV, which cause the majority of cervical and anal carcinomas and a subset of other genital and oropharyngeal cancers. The quadrivalent vaccine also includes VLP of the mucosal low-risk HPV6 and 11, which induce 90% of genital warts. However, these vaccines may not protect against infections with over 20 less prevalent mucosal or cutaneous HPV. Immunizations with peptides of minor capsid protein L2, in contrast, induce low-titer but cross-protective anti-L2 antibodies though L2 contains type-common motifs, crucially involved in early viral infection. L2 is immunologically subdominant in the context of native virions or co-assembled L1-L2 VLP. To improve immunogenicity of L2 we have generated chimeric RG1-VLP by genetic insertion of the cross-neutralizing L2 epitope into RG1 within the immunogenic L1-L2 DE surface loop of HPV16, resulting in a 360-fold display of the RG1 particle by the assembled particles. Immunization with RG1-VLP using human adjuvant adjacent to the mucosal L2 induces a strong immune response against RG1 and RG1-L2, but not against low-risk HPV6/11/31/45/52/58/63/15/36/45/56/66/73, mucosal low-risk HPV6/11/32/44/40/47/73, common cutaneous HPV2/7/17/31, and gena-beta HPV1-8 but not HPV1/4 independent of MD2, a putative cell surface. We have used this adjuvanted link between VLP immunization, replication and epithelial stem cells. Using VLP-infected lesional skin we demonstrated that VLP induce keratinocyte cell marker, keratin 15, in follicular epithelium and granular and suprabasilar epidermis. To confirm the relationship between K15 expression and VLP replication we infected immortalised (NTERT) and primary keratinocytes with VZV. We observed that VZV colocalises with the stem cell marker, keratin 15, in follicular epithelium, sweat gland and suprabasilar epidermis. To confirm the relationship between K15 and viral infection we depleted K15 using siRNA and infected cells with VZV. Depletion of K15 reduced the expression of immediate early and late markers of viral replication by qPCR and Western blotting. K15 stimulation by vitamin D treatment increased viral infection and replication as well as increasing expression of the Wnt inhibitor, Dickkopf 1. Furthermore, mechanisms by which K15 regulate proliferation and identification of novel targets for the therapy of inflammatory acne vulgaris.

640
NLRP3-inflammasome activation by Propionibacterium acnei is a pivotal event in inflammatory acne


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Acne vulgaris (AV) is a frequent potentially severe inflammatory skin disease that affects 85% of people age 12-24 and is characterized by comedones and inflammatory papules, pustules, and nodules. The pathogenesis of this disease is multifactorial, with inflammatory lesions thought to be triggered by proliferation of Propionibacterium acnei (P. acnes) in the anagenic environment of the hair follicle and involving activation of innate immune responses, such as those involving Toll-like receptor 2 (TLR2). Here we identify the molecular mechanism by which P. acnes activates an innate immune response resulting in vivo in neutrophil-rich skin inflammation characteristic of inflammatory acne. Using a transgenic AV mouse model, we show that P. acnes is a strong trigger of the neutrophil-rich inflammatory macrophage IL-1β, IL-18 and IL-6 secretion in vivo, as well as IL-1β production in the skin in vivo. Exposure of monocytes to P. acnes led to NLRP3-inflammasome activation, caspase-1 cleavage and processing of the mature form of IL-1β. This is dependent on phagocytosis, subsequent lysosomal destabilisation, ROS production, and cellular Ki+ efflux. Consistent with this, in mice P. acnes induces neutrophilic skin lesions reminiscent of acne in NLRP3-inflammasome-, IL-1β-dependent-, and TLR2-independent experiments that require the presence of active P. acnes. To further investigate the biology of this mucosal mucosal mucosal mucosal infection with P. acnes, we depleted K15 using siRNA and infected cells with VZV. Depletion of K15 reduced the expression of immediate early and late markers of viral replication by qPCR and Western blotting. K15 stimulation by vitamin D treatment increased viral infection and replication as well as increasing expression of the Wnt inhibitor, Dickkopf 1. Furthermore, mechanisms by which K15 regulate proliferation and identification of novel targets for the therapy of inflammatory acne vulgaris.

641
Functional properties of dermal dendritic cells

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Skin dendritic cells (DCs), sentinel of the skin immune system, are potent antigen presenting cells and have the ability to stimulate T cells. Until now, it has been difficult to attribute a specific function to the distinct skin DC subsets, in response to pathogens or peripheral tolerance mechanisms. We propose to use XCR1 as a unique marker for cross-presenting DCs and as a target for protective immunization against pathogens or cancer cells.

642
Keratin 15 and VZV replication in skin

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Varicella-zoster virus (VZV) primarily infects epithelial tissue, replicating within differentiating keratinocytes. Latent infection occurs in sensory ganglia and reactivated VZV initially infects the sensory ganglia and suprabasal epidermis. To confirm the link between K15 expression and viral infection we depleted K15 using siRNA and infected cells with VZV. Depletion of K15 reduced the expression of immediate early and late markers of viral replication by qPCR and Western blotting. K15 stimulation by vitamin D treatment increased viral infection and replication as well as increasing expression of the Wnt inhibitor, Dickkopf 1. Furthermore, mechanisms by which K15 regulate proliferation and identification of novel targets for the therapy of inflammatory acne vulgaris.

643
Metal allergens nickel and cobalt facilitate human Toll-like receptor 4 homodimerisation independently of MD2

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Development of contact allergy requires cooperation of adaptive and innate immunity. Nickel (Ni2+) stimulates innate immunity by activating human Toll-like receptor 4 (TLR4), the receptor for bacterial lipopolysaccharide (LPS). The mechanism likely involves receptor dimerization. Yet, direct experimental evidence for metal-induced TLR4 dimerisation is pending and the exact role of the TLR4 co-receptor MD2 is unclear. Moreover, the contribution of TLR4 to proinflammatory activation by related metal haptons is unknown. Here, we reveal cobalt (Co2+) as second metal allergen activating TLR4/MD2 and confirm a crucial requirement of the non-conserved metal-binding hotspots H546/H548 in HLR4 for Co2+ -induced proinflammatory activation. Experiments with a novel hTLR4/MD2 mutatis mutandis established establishment of dimerisation as mechanism of metal- and LPS-induced hTLR4 activation. Yet, in interaction studies LPS required MD2 for dimerisation whereas Co2+ induced TLR4 dimerisation independent of MD2. These findings suggest that infantile expression of TLR4 in the skin could be involved in the development of metal allergy.
CD40L induces an antimicrobial pathway against intracellular infection in human mono-
cytes

The skin-specific NKG2D ligand H60c directly triggers cytotoxicity in epidermal γδ T cells

Delayed permeability barrier repair in beta-defensin 14 deficient mice

Inflammatory reaction induced by streptococcus pyogenes group A-stimulated keratinocytes in an in vitro keratinocyte model of infection

CD40L induces an antimicrobial pathway against intracellular infection in human mono-
cytes

Inflammation-induced beta-defensin 14 protects mice from lethal herpes simplex virus infec-
tion but not M. tuberculosis
650 Modulation of innate tetherin antiviral activity in mature Dendritic Cells can restrict HIV replication and transfer
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Dendritic cells (DC) located at mucosal surfaces are among the first immune cells to encounter disseminating pathogens. The restriction factor tetherin (also known as CD317, BST-2 or HM1.24) has been shown to potently restrict HIV-1 infection by retaining viral particles at the cell surface. We report here that basal tetherin expression in Myeloid (MDC) and Monocyte-Derived DC was upregulated by IFN-α treatment. In contrast to Hela or 293T cells, infectious viral release in DC was only modestly reduced in the absence of Vpu compared to wild-type viruses. Strikingly, immunofluorescence analysis revealed that tetherin was excluded from HIV-containing Tetherin-Enriched Micropod (TEM) even when cells were pre-treated with IFN-α. In contrast, in LPS-mediated mature DC, there was a dramatic tetherin re-localization to the TEM in mature DC. LPS-induced localization of tetherin in TEM correlated with an increase in restriction of DC-mediated HIV-1 transfer to CD4+ T cells. In conclusion, we unravel that HIV replication in DC and spread toward target cells by-passing tetherin restriction could however be overcome with TLR4 agonists restoring an efficient tetherin-mediated innate antiviral restriction.

652 A role of transcriptional factor PU.1 in the gene expression of dendritic cells
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The MHCI class II antigen presenting cells express antigen peptide and regulates activation of the T cell through TCR. Activation of T mediated by TCR/MHCI class II interaction involves in several immune-related diseases including atop dermatitis, and contact hypersensitivity. However, the mechanism of expression of the MHCI class II on dendritic cells (DC) is still obscure. We have previously found that siRNA treatment of a transcription factor PU.1 recovered contact hypersensitivity in mice. This observation prompted us to analyze the role of PU.1 in MHCI class II expression in dendritic cells. We performed a reporter assay, an electrophoretic mobility shift assay (EMSA), and a chromatin immunoprecipitation (ChIP) assay to evaluate the effect of PU.1 in transcription of MHCI class II. We have been mouse-derived DC, EL4. Effect of PU.1 on MHCI class II promoter function and its gene expression were also analyzed by above-mentioned methods in addition to quantitative PCR and FACS with using PU.1 siRNA. ChIP assay revealed that PU.1 constitutively bound to a putative promoter region (−1000) independent from repressing conditions, whereas histone acetylation status of the CITAPD was dramatically down-regulated by LPS-stimulation, resulting in significant decrease transcription of MHCI class II and CITAPD. Reporter assay and EMSA showed that PU.1 trans-activated the CITAPD through direct binding via PU.1 binding sequence located at just upstream of transcription initiation site. Knock-down of PU.1 by siRNA resulted in down-regulation of CITAPD transcription, histone acetylation of the CITAPD, cell surface expression of MHCI class II and T cell stimulation activity. PU.1 plays critical roles in MHCI class II expression as transcription factor and epigenetic regulator.

653 Toll-like receptor genes polymorphism in atopic dermatitis patients
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One of the dominant pathological point of atopic dermatitis (AD) was high frequency of skin infections caused by Staphylococcus aureus. The main cause of such situation was the dysfunctions of skin innate immune mechanisms, such as decreased expression of antibacterial peptides in the skin, decreased of cellular defence mechanisms caused by domination of TH2 lymphocytes and disturbances of structure and function of toll like receptors (TLR) cellular proteins which recognize bacterial products. The aim of our study was to analyze the association between atopic dermatitis frequency and polymorphisms in TLR-2, 4 and 9 genes. Material and methods 203 patients with atopic dermatitis and 171 healthy persons were included in the study. From each person 5 ml peripheral blood were collected, genomic DNA were isolated and using PCR based methods polymorphism of TLR genes were determined. Three polymorphism were analyzed: +869 AG of TLR-4 gene, −323 CT of TLR4 gene and Arg753Gln of TLR-2 gene. The results were statistically analyzed using p2 Pearson test. Results We have found, that in comparison to the healthy controls, the patients with AD more frequently have polymorphic variant of Gln 753 of TLR-2 gene that encodes nonfunctional form of its receptor. 10,8% of AD patients with AD more frequently have polymorphic variant of Gln 753 of TLR-2 gene that encodes nonfunctional form of its receptor (10,8% vs 4,7%, p=0,02). The presence of this allele increased the risk of AD (OR=2,3; 95% CI (0,97-5,29); p=0,039. We have found no differences in the frequency of alleles and genotypes of TLR-4 and TLR-9 genes polymorphisms in both compared groups. Conclusion Our results suggests, that TLR-2 gene variant coding nonfunctional receptor could play the role in the pathogenesis of atop dermatitis.

654 A comparison of the innate immune responses of keratinocytes to probiotics and skin com- mensals
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Probiotics have been defined as “Live microorganisms which when administered in adequate amounts confer a health benefit on the host”. Studies in the gut have demonstrated that probiotic bacteria modulate innate immune responses in epithelial cells. Previously, we demonstrated the potential of probiotic bacteria as therapeutic agents for skin, particularly in the prevention of infection. In the current study, we have investigated the effect of two probiotic strains, L. reuteri ATCC 55730, and L. rhamnosus, on the innate immune responses of keratinocytes. For comparison, the responses elicited by a skin pathogen, S. aureus, and a skin commensal, S. epidermidis were also investigated. Normal human epidermal keratinocytes (NHEK) were exposed to 10-100 chlam of each of the probiotics, 10-100 chlam of S. aureus, and 10-100 chlam of S. epidermidis for 12 hours. Additionally, NHEK were pre-exposed to either the probiotics or S. epidermidis for 1 hour prior to infection with S. aureus for 12 hours. Toll-like receptor 2 gene expression and IL-8 production were measured using qRT-PCR and ELISA. A TLR-like receptor 2 mRNA expression was significantly increased in NHEK pre-exposed to S. epidermidis prior to S. aureus infection (P<0.009) while none of the other bacteria induced any significant change in TLR2 mRNA production. There was a non-significant trend toward a decrease in IL-8 production by NHEK in response to S. aureus in ELISA assays. In conclusion, we suggest that S. epidermidis may act to prime NHEK against S. aureus infection by upregulating TLR2 expression in the presence of S. aureus. Conversely, this does not appear to be the case with either of the probiotics used. However, L. reuteri improved S. aureus clearance from skin wounds through production of the neutrophil chemoattractant, IL-8.

655 Aryl Hydrocarbon Receptor (AhR) Ligands Are Detected In Skin Scales Of Malassezia-Associated Diseases
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We have previously shown that Malassezia yeasts produce in vitro potent AhR hydrocarbon receptor (AhR) ligands such as indirubin, indole-3,3-carbazole (ICZ), 10-formylindole-3,3-carbazole (FICZ), malassezin and pityriacitrin. This property is widespread in Malassezia species yet in the species M. furfur it has been associated with seborrhoeic dermatitis (SD) and pityriasis versicolor (PV) isolates. Our aim is to evaluate skin scale extracts from diseased (PV and SD) and healthy volunteers for their AhR inducing activity and the existence of the aforementioned substances. Seven patients in SD, 3 PV and 3 healthy volunteers were included in this study. Initially the skin extracts from the 10 volunteers were evaluated for AhR inducing activity in HepG2 cells transfected with a luciferase reporter gene. In a next step, to further relate this activity to Malassezia metabolites, the same skin extracts were investigated with LC/MS/MS. The skin extracts from patients showed 1000 times stronger AhR inducing activity than the skin extracts from the healthy volunteers. Interestingly the extracts showed no activity in a relevant mouse cell line. The presence of FICZ, ICZ, malassezin, indirubin and pityriacitrin in the skin extracts was confirmed by LC/MS/MS. The same compounds have also been identified in M. furfur extracts. Substances like malassezin have been indentified solely from this yeast and are considered chemical signatures. These findings support the Malassezia origin of these potent AhR ligands on the skin. Our findings demonstrate for the first time the presence of potent AhR ligands on the skin and relate them with certain Malassezia-associated diseases. Furthermore, they underscore this yeast as the source for their in vivo production and confirm Malassezia implication in skin diseases as SD.

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656 Human skin explants: a model to study the interaction between epidermal cells and the cutaneous microbiota
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The skin represents an optimal habitat for a wide range of microorganisms. So far, a large number of studies have been devoted to investigate the interaction between bacteria and cutaneous cells in order to elucidate the role of the epidermal microbiota in the physiology of the skin. Most of them have been carried out using keratinocyte cell lines or animals as model to conduct the research.

657 The aim of the present work was to investigate the molecular response to microbial stimuli of a healthy human skin explant and to determine the expression of human skin malodour generation.

658 To return after the withdrawal of antibiotics the development of novel antimicrobial therapies based on AMPs seems to be realistic. The purpose of this study was to synthesize a group of antimicrobial peptides and investigate their antimicrobial activity against plantonic cells and biofilms formed by clinical isolates of S. aureus, including strains resistant to antibiotics. Biofilms were cultured on polystyrene surfaces for 1, 2 and 3 days. The susceptibility of structures to conventional antibiotics (i.e. vancomycin, fusidic acid, mupirocin, vancomycin). Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined. Both techniques clearly designated the existence of two distinct genotypes.

659 Emotional stress induces the expression of pro-inflammatory cytokines IL-1, IL-6 and IL-8 in HaCaT keratinocytes
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We observed S. aureus mainly induced the expression of human β-defensins in cultured karatinocytes stimulated by the secretion of human β-defensins. The expression of human β-defensins is induced by NO2D at a quite similar intensity in fetal as well as in adult skin, while neither dermal cell precursors in prenatal skin nor dermoeic cells in adult skin show reactivity for these markers. The nosological skin cultures revealed a similar differential potential of fetal, neonatal and adult keratinocytes with regard to histology and differentiation marker expression. Also the immunoreactivity of TLR 1 and 6 as well as NO2D was comparable in all three age groups. Activation of keratinocytes with the synthetic TLR3 ligand poly I:C, mimicking viral ds RNA, induced high secretion of CCL3 and CCL4 as well as IFN-inducible cytokines (e.g. CXCL9-11) in fetal keratinocytes suggesting that already prenatal skin possesses an effective antiviral innate defense program.

660 MALP2 regulates the SCF/FADS2 proinflammatory signalling in human S295 sebocytes
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In previous studies, steam-GA desaturese (SCD) was associated with the conversion of the essential FA linoleic acid to the proinflammatory-arachidonic acid. Moreover, it was shown that SCD is a key enzyme for the synthesis of the essential FA linoleic acid.

661 Activity of antimicrobial peptides: camel, citrumin, protegin, temporin A and lipopeptide against biofilms formed by clinical strains of Staphylococcus aureus and their safety profile on HaCaT keratinocytes
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The therapy of staphylococcal skin infections is facing several difficulties caused essentially by the development of resistant strains and the growth of staphylococcal biofilms. Being in mind that topical treatment is often difficult or even impossible to use, we decided to return after the withdrawal of antibiotics the development of novel antimicrobial therapies based on AMPs seems to be realistic. The purpose of this study was to synthesize a group of antimicrobial peptides and investigate their antimicrobial activity against plantonic cells and biofilms formed by clinical strains as well as to determine their safety on HaCaT keratinocytes. We have examined 5 antimicrobial peptides: camel, citrumin, protegin, temporin A and lipopeptide. The antimicrobial activity of the examined peptides do not exert any toxic effect on HaCaT cells in their antimicrobial concentrations. The most promising peptides seem to be citrumin and temporin, as they are toxic only in two high concentration (100 and 1000 μg/mL), with relatively low MIC values.
662 Sphingosine-1-phosphate and TLR2 signalling enhance inflammatory cytokine production and cell migration in human dermal fibroblasts
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The lysophospholipid sphingosine-1-phosphate (S1P) is a key regulator of diverse biological activities such as inflammation, cell migration, and wound healing. Most of the effects of S1P are mediated by five distinct G protein-coupled receptors (S1PR1-5). Recent studies suggest that cross-talk between S1P receptor (S1PR) and Toll-like receptor (TLR) signalling pathways differentially modulates immune responses. Previously, we have shown that TLR2/1 and TLR2 ligation lead to increased production and secretion of pro-inflammatory cytokines in normal human dermal fibroblasts (NHDF), whereas a small increase is observed after stimulation with TLR3, TLR4 and TLR9 agonists. The aim of the present study was to elucidate the interaction between S1PRs and TLRs in NHDF in inflammation and cell migration. NHDF express S1PR1, S1PR2 and S1PR3, as shown by quantitative real-time RT-PCR. Stimulation with S1P resulted in a time- and concentration-dependent increase of IL-6 and IL-8 mRNA expression and secretion. Pro-inflammatory cytokine production was markedly increased when Poly(A),U, LPS or CpG ODN on the one hand, and ATP on the other hand were used for stimulation. Moreover, S1P co-stimulation with ATP significantly increased the production of IL-1β, IL-6 and IL-8. ATP induced TNF-α production was increased in the presence of S1P and vice versa. The differential secretion of cytokines and chemokines in fibroblasts is crucial for cutaneous immunity. Our results suggest that cross-talk between S1P and TLR signalling pathways may have an important role in regulation of dermal immune responses and cell motility.

663 Critical role of cytokine environment for TLR-ligand induced DC phenotypes
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Vaccination against infection or cancer aims to establish effective immune responses, however, especially tumor immunotherapy still awaits further improvement. Dendritic cells (DC) are critical in the transition of innate immune signals to adaptive immune responses and therefore for successful immunizations. Clinical trials directly applying DC as well as adjuvants inducing DC activation generated divergent results. It is well known that Toll-like receptor ligands (TLR-L) act as adjuvants leading to DC maturation and DC cytokine production such as pro-inflammatory IL-12 or anti-inflammatory IL-10. The cytokine environment during DC activation represents an additional level of DC regulation that has not been well characterized in combination with the other signals. To this end, we investigated the influence of important stromal and developmental cytokines, TGF-β, IL-4 and GM-CSF on the development of DC activation and phenotypes. Activation of DC with TLR-L led to upregulation of MHC II, CD80 and CD86 independent of IL-4 and GM-CSF but was reduced by TGF-β. Differences were seen in DC cytokine production depending on both cytokine environment and TLR-L: IL-4 and GM-CSF led to amplification of pro-inflammatory IL-12-p70 production induced by LPS (TLR4-L), imiquimod (TLR7) and CpG (TLR9-L) while TGF-β failed to do so. Further stabilization of an inflammatory DC phenotype was achieved by inhibition of anti-inflammatory IL-10 induced by these TLR-L by IL-4 or GM-CSF. In contrast, no synergistic or additive effect was observed after co-stimulation with poly(A),U, LPS or CpG ODN, respectively. Next, we investigated the influence of IL-1 and GM-CSF on the cytokine production of recombinant human DC. Phase II studies after topical application of a DC vaccine in melanoma patients are ongoing, which may have important role in regulation of dermal immune responses and cell motility.

664 Role of the extra-cellular danger signal adenosine-5'-triphosphate on the 1-fluoro-2,4-dini- trobenzene-induced maturation of THP-1 cells
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The identification of the innate immune and cellular stress response pathways triggered by contact allergens has been a major focus in the last years. Allergens are able to induce a variety of immune responses such as inflammation, cell migration, and wound healing. Most of the effects of S1P are mediated by five distinct G protein-coupled receptors (S1PR). Notably, S1P, ATP and cytokines are involved in the control of immune and inflammatory responses. The interface between S1PRs and TLR2 signalling pathways differentially modulates immune responses. We have shown that S1P and ATP act in a concentration-dependent manner. Similarly, TLR2 activation increased fibroblast migration, which was further enhanced in the presence of S1P. Collectively, these findings indicate that cross-talk between S1PRs and TLR2 is crucial for cutaneous immunity. Our results suggest that cross-talk between S1P and TLR signalling pathways may have an important role in regulation of dermal immune responses and cell motility.

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665 Response of normal human keratinocytes to Demodex mites
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Human Demodex mites are regarded by most investigators as commensal organisms, occupying two niches in the pilosebaceous unit of man and cause no adverse effect to the host. The role these mites play in the bio-balance of the skin has been largely ignored. As a result there is no satisfactory conclusion on what role these mites may play in human skin diseases. Their numbers are increased in papulopustular rosacea, where an aberrant innate immune response is suggested to induce the clinical features. In this study we sought to evaluate the potential of live Demodex mites to mediate an inflammatory response. Live Demodex mites (n=25) were extracted by gentle manipulation from skin surface biopsies by fine forceps and co-cultured for 24 h and 48 h with normal human keratinocytes (NHK). The response to live Demodex mite stimulation by NFκB was assessed by employing Human Toll-Like Receptor Signaling Pathway PCR Arrays evaluating 84 genes central to TLR-mediated signal transduction and innate immunity. Multi-Analyte ELISA Arrays were utilised to evaluate human inflammatory cytokines and chemokines. In this study we found a significant increase in mRNA levels of TLR1 at 24 h and 48 h, TICAM1 at 48 h. On a protein level at 24 h pro-inflammatory cytokines IL-1β and IL-6 were increased in control cells but did not reach significance. At 48 h there was a significant increase in GM-CSF mRNA levels. These results indicate Demodex mites have the potential to promote inflammation and this may be through a TICAM1-dependent pathway.

666 Propionobacterium acneus induces a dose-dependent activation of cultured human keratinocytes
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Different external stimuli induce coordinated signaling events in keratinocytes, and this ability is crucial for these cells to discriminate between friend and foe. Upon these changes keratinocytes get activated, and the cells need to make decisions how to respond to the detected stimuli. When the skin microbiota is altered an altered state will lead to the development of skin diseases. Earlier we have investigated if similar processes also play a role in acne pathogenesis, and followed the mRNA expression changes of selected genes (IL-1α, TNF, TGFα, K6, K16, ICAM1, VACM1) implicated in keratinocyte activation in immunized keratinocytes (HPK-KEV) in response to Propionibacterium acneus (P. acne) treatment. We concluded that the bacteria is indeed capable of the initiation of the activation program which may play a role in the pathogenesis of acne. Currently we are analyzing if the activation is dose dependent by treating HPK-KEV cells with the P. acne 889 strain at different multiplicity of infection (MOI=25, 50, 100, 200, 1000), and study the corresponding mRNA expression changes of TNFα, IL-1α, and the negative regulator of NF-κB signalling TNAPF3. The mRNA expression of the genes increases dose dependently in the 6-hour samples. These data appear to be transient using MOI25 (25, 50, 100), whereas at high MOIs (200, 1000) they stay elevated until the 24 hour samples. Under these conditions the P. acne 889 induces increased pro-inflammatory responses. Whether these effects are in a specific is currently being investigated. These suggest that P. acne is capable of the induction of keratinocyte activation, but its extent depends on the applied strain and the bacterial load. The studied pathogenic strain (889) at high loads induces atypical keratinocyte functions (hyperproliferation, abnormal differentiation) also observed in vitro in acne pathogenesis.

667 The ability of components in NZ honeys to stimulate monocytic cell lines and primary blood monocytes may influence honeys ability to aid wound healing
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The process of wound healing is a complex physiological and biological process involving a series of events including chemical, cellular and biological events involving both degenerative and reparative phases. Medical honey used for wound healing has well-established anti-microbial properties, and now pro-inflammatory and immunomodulatory effects are reported as well. We have for the first time identified honey-derived arachidonic acid proteins (APs) as a new pro-inflammatory bioactive that stimulates TNF-α expression by monocytes. NZ kanuka honey seems to have superior ability to stimulate monocytic cell lines and primary blood monocytes to produce TNF-α, a factor which promotes early stages of wound healing, and increases wound strength. Agar diffusion and rocket electrophoresis assays were developed to measure AGP content. As proof-of-principle they were used to measure the levels of AGPs in different commercial honey samples. The amount of AGPs in different honeys varies significantly in the 6-hour samples. These data appear to be transient using MOI25 (25, 50, 100), whereas at high MOIs (200, 1000) they stay elevated until the 24 hour samples. Under these conditions the P. acne 889 induces increased pro-inflammatory responses. Whether these effects are in a specific is currently being investigated. These suggest that P. acne is capable of the induction of keratinocyte activation, but its extent depends on the applied strain and the bacterial load. The studied pathogenic strain (889) at high loads induces atypical keratinocyte functions (hyperproliferation, abnormal differentiation) also observed in vitro in acne pathogenesis.
668
Interferon γ Increases Toll-like Receptor 2 Expression in Primary Human Keratinocytes in a Concentration Dependent Manner
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The innate immune system supports the physical skin barrier in antimicrobial defense. Human keratinocytes constitutively express various members of the toll-like receptor (TLR) family important for innate immunity including TLR2, TLR4 and TLR5. TLR2 recognizes pathogen associated molecular pattern of gram-positive bacteria such as lipoteichoic acid. TLR4 recognizes lipopolysaccharide, a cell wall component of gram-negative bacteria. The ligand of TLR5 is flagellin, a protein expressed in flagellated bacteria. We asked whether TLR2, TLR4 and TLR5 expression is stimulated by cytokines in human keratinocyte culture in vitro. The expression of human TLR2, TLR4 and TLR5 was assessed by real-time PCR analysis and subsequent gel electrophoresis for verification. For stimulation experiments we used cytokines which have been described to be involved either in Th1 or Th2 dominated immune responses: interferon (IFN)γ, IL-4, IL-5, IL-6, IL-13, IL-15, IL-17, IL-18, IL-20, IL-21, interferon (IFN)γ, tumor necrosis factor (TNF)α and tissue derived growth factor (TGF). We found a significant upregulation of TLR2 expression in keratinocyte cell culture especially by stimulation with IFNγ. The effect of IFNγ stimulation was concentration dependent with a maximum response after stimulation with 40 nm/ml. TLR4 expression was not stimulated by the cytokines tested. As IFNγ is a type-1 cytokine it may be responsible for the induction of Th2 expression seen in psoriatic skin.

669
Suscitability of bacteremia derived Staphylococcus aureus strains to different epithelial antimicrobial proteins
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Staphylococcus aureus is a major human pathogen and the most important cause of life-threatening bacterial infections like bacteremia and sepsis. These infections are often caused by S. aureus derived from the own epithelial microflora. Antimicrobial proteins (AMP) such as human beta-defensin-3 (hBD-3) and RNase 7 protect the skin and other epithelia against infections and are highly active against staphylococci. The aim of this study was to determine whether S. aureus strains from sepsis patients are less susceptible against various antimicrobial proteins than strains derived from age and sex matched S. aureus colonizers (control strains). S. aureus strains derived from bacteremia patients (n = 14) as well as control strains were tested in parallel for their susceptibility to different AMP (hBD-3, RNase 7, psoriasin, lysozyme) by agar diffusion assay and microdilution assay. In only two cases, the sepsis strains were less susceptible to hBD-3, RNase 7 and psoriasin compared to the S. aureus strains derived from S. aureus colonizers. In the remaining cases, the S. aureus strains from sepsis patients showed equal or even more susceptibility to the tested AMP. In summary, our data indicate that S. aureus strains causing sepsis are not more susceptible to the investigated AMP than S. aureus strains derived from healthy S. aureus colonizers. Although we can not exclude a potential relevance of other AMP not tested here, our results suggest that the onset of sepsis may not be linked to a different susceptibility of S. aureus to skin-derived AMP.

670
Vitreoscilla filiformis extract decrease pruritic mediators involved in skin disease
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Seborrhoeic (SD) and atopic dermatitis (AD) are frequent chronic inflammatory skin diseases characterized by pruritic flares subsequently leading to eczema, erosions and/or scaly lesions. Intense pruritus and scratching are important initiating/aggravating factors in the pathophysiology and maintenance of SD and AD. In both, dysregulated innate and adaptive immunity as well as infectious agents play a role in disease pathophysiology and lead to serious impairment in the patient’s quality of life. The interactions between sensory nerves, keratinocytes, activated endothelial cells and skin-infiltrating immune cells are crucial for pruritus induction by the release of neuropeptides, amines, prostaglandins, serotonins and cytokines. Thus, compounds capable to inhibit secretion of inflammatory and/or pruritic mediators may have a beneficial role in SD and AD. Vitreoscilla filiformis extract (VF) has been demonstrated to exert anti-inflammatory and moisturizing capacities by a poorly understood mechanism. Therefore, the aim of this study was to examine the impact of VF extract on down regulate gene expression and release of inflammatory and pruritic mediators from AD cells. In vitro, VF was able to down regulate gene expression at the transcriptional level and release of important inflammatory and pruritic mediators in all cells after LPS, LTA, TNF or IL-31 stimulation. For example, VF directly inhibits generation of TSLP and IL31, both key cytokines involved in pruritic disease. These results bring some new information about the mechanism of action of this bacterial extract that will be confirmed in clinical trial.