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

G Nina,[1] C Walker,[2] J Harder,[1] W Müller[2] and E Proksch[1] *1 Dermatology, University of Kiel, Kiel, Germany and 2 Faculty of Life Sciences,, University of Manchester, Manchester, United Kingdom*

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 was induced by tape stripping and the repair was monitored as recovery in TEWL. Recombinant mBD-14 protein or the vehicle was applied topically on back skin of mBD-14 deficient mice after barrier disruption. Inflammation, epidermal proliferation, thickness, and differentiation were monitored by (immuno)-histology. mBD-14-deficient mice exhibited 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-1beta 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 epidermal 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 chemoattractant activity of this defensin. It was shown that mutated mBD-14 has a reduced chemoattractant activity. In conclusion, mBD-14 expresses pro-inflammatory activity which is important for skin barrier repair.

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Studying the negative regulatory factors of the *Propionibacterium acnes*-induced signaling pathways in *in vitro* cultured immortalized keratinocytes

L Erdei,[1] G Tax,[1] BS Bolla,[1] E Urbán,[2] L Kemény[1] and K Szabó[1] *1 Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary and 2 Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary*

Propionibacterium acnes (*P. acnes)* is a commensal bacterium, but it can also activate different pathogen recognition receptors (e.g Toll-like receptors; TLRs), and thus induce innate immune and inflammatory events in human epidermal keratinocytes. These molecular pathways are well characterized, but little is known about the endogen negative regulatory mechanism that may control these events, and counteracts the TLR-induced signal transduction pathways. In order to identify and analyze endogen factors playing a key role in the attenuation of the *P. acnes*–induced TLR activation, we studied the mRNA and protein expression of selected negative regulators of these signaling events (SIGIRR, TOLLIP, TNFAIP3, TNIP1) in a human immortalized keratinocyte cell line (HPV-KER) in response to the bacterial treatment by real time RT-PCR and western blotting. Our results show that all the investigated negative regulators were expressed in HPV-KER cells. Moreover, the TNFAIP3 and TNIP1 mRNA expressions significantly, and dose dependently increased in response to the bacterium. Next, we studied the effect of various *P. acnes* strains (*889, 6609)* belonging to different phylogenetic groups within the species, but no major differences have been observed in the induced expression changes. By monitoring these factors at the protein level by western blotting we found increased TNFAIP3 and decreased SIGIRR expressions following the bacterial treatment, and these events also appeared to be dose dependent. Our study suggests that in our *in vitro* model system *P. acnes* causes the dose-dependent activation of downstream TLR signaling processes. Specialized, endogen negative regulators do exist in these cells which may control the bacterial-induced molecular events, and thus can be important for the maintenance of epidermal homeostasis.

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Sulfasalazine and thalidomide inhibit extracellular trap formation by human neutrophils

AM Lin, R Khandpur, M Riblett and A Bruce *Dermatology, Univerisity of Michigan, Ann Arbor, MI*

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 ETosis 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 insufficient clearance 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 ETosis 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 these drugs may be attributed in part to their inhibition of ETosis. 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, lupus, 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.

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

A Bruhs, T Schwarz and A Schwarz *Department of Dermatology, University of Kiel, Kiel, Germany*

Beta-defensins are antimicrobial peptides (AMP) of the innate immune system produced in the skin by various stimuli, including proinflammatory cytokines, bacterial infection and UV radiation (UVR). Using the contact hypersensitivity model, we recently demonstrated that the UVR-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 intravenously 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 by a decreased cellular infiltration in the spinal cord 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.

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The effect of the skin microbiome on the barrier properties of *in vitro* cultured immortalized keratinocytes

SB Bolla,[1] G Tax,[1] L Erdei,[1] E Urbán,[2] L Kemény[1] and K Szabó[1] *1 Dermatology and Allergology, University of Szeged, Szeged, Hungary and 2 Clinical Microbiology, University of Szeged, Szeged, Hungary*

The skin microbiome has been suggested to have direct effects on various molecular and cellular properties of the human cells. Our aim was to analyze whether the *Propionibacterium acnes* (*P. acnes*) bacterium has any effect on the barrier properties of the epidermis. For that, HPV-KER cells (human immortalized keratinocytes) were treated with various *P.acnes* strains (*889, 6609, ATCC 11828*) in different doses, and changes in the barrier properties of a keratinocyte monolayer were analyzed in real time using the xCELLIgence system. *P. acnes* induced dose-dependent barrier changes, where the effect of the pathogenic *889* and *ATCC 11828* strains appeared to be more robust. We also analyzed the effect of the bacterium on the mRNA expression changes of known tight junction proteins claudin 1, 2, 4 (CLDN1, 2, 4), ocludin1 (OCL1) and ZO1 in confluent HPV-KER cultures using real time RT-PCR. CLDN1, OCL1 and ZO1 mRNA-s were present in these cells, and the expression of CLDN1 decreased, whereas ZO1 levels increased in response to the bacterial treatment. *P. acnes* can also induce innate immune and inflammatory events in keratinocytes. Thus, we studied if TNFa pro-inflammatory cytokine treatment (1, 5, 10 ng/ml) can modify the barrier properties of the HPV-KER monolayer. We found that TNFα, and thus inflammatory conditions may loosen the epidermal barrier, possibly leading to the easier penetration of keratinocyte-, as well as bacterially-derived factors to deeper tissue compartments, thus leading to more severe acne symptoms. These findings suggest that the skin microbiome can modulate the barrier properties of the human skin, and these events can have important role in the pathogenesis of different skin diseases.

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The human skin immune atlas: Three-dimensional reconstruction of serial histology and computational image analysis of dermal immune populations in normal skin

A Hald,[1] W Yu,[2] B Roediger,[3] W Weninger[3] and PL Tong[3] *1 Department of Skin Neoplasia Pharmacology, LEO Pharma, Ballerup, Denmark, 2 Central Imaging Facility, Institute of Molecular & Cell Biology (IMCB), A*STAR, Singapore, Singapore and 3 Immune Imaging Program, Centenary Institute, Camperdown, NSW, Australia*

Histopathology is an established and reliable approach to analyze and diagnose various skin diseases. The information, however, is confined to two-dimensions and may be prone to sectioning and staining artifacts. Accurate three-dimensional *in vivo* examination of skin has largely been dependent on fluorescent transgenic murine model systems. It is paramount that these studies are validated in human skin as well. The advent of automated and reliable staining protocols with slide-scanning technology permits generation of large amounts of digital images from histology for two-dimensional examination. Three-dimensional (3D) reconstructions of these acquired sections and its subsequent analysis have significant potential to enhance our understanding, for example those involving spatial and temporal change of cellular and vascular structures *in situ*. Assessment of tissue samples in a 3D space is important, however reconstruction of histology stacks can be challenging. Some of the difficulties encountered include linear/non-linear displacement between the slices and color/brightness variations. In addition, the quality of traditional histology is operator-dependent and may introduce artifacts hindering subsequent image analysis. We have developed a reconstruction platform and include technology for sample preparation, high-resolution image acquisition and automated computational reconstruction. The 3D location of innate and adaptive immune cells in human skin is presented including proximity to anatomical structures. This study confirms the utility of 3D histological reconstruction in assessment of normal and diseased skin to generate hypotheses in pathomechanisms of inflammatory skin conditions as well as drug delivery applications.