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. Inflammation 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 found after barrier repair when 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 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 differentiation 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 chemotactically active of this defensin. It was shown that mutated mBD-14 has a reduced chemotactic activity. In conclusion, mBD-14 expresses pro-inflammatory activity which is important for skin barrier repair.

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 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 inappropriate and 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 of these drugs may be attributed to 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 were able to show that several drugs may inhibit ETosis. In conclusion, 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.

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

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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 immunomodulated 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 expressions changes of known tight junction proteins claudin 1, 2, 4 (CDL1N, 2, 4), occludin (OCL1) and ZO1 and confluent HPV-KER cultures using real time RT-PCR. CDL1N, OCL1 and ZO1 mRNA were present in these cells, and the expression of CDL1N 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 TNFα pro-inflammatory cytokine treatment (1, 10 ng/ml) can modify the barrier properties of HPV-KER cells. However, we found that TNFα does not 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 skin, and these events can have important role in the pathogenesis of different skin diseases.

The effect of beta-defensin 14 on the expression of pro-inflammatory mediators in neutrophil extracellular traps

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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 endogenous negative regulatory mechanism that may control them. We studied the endogenous negative regulatory mediator that can control TNF-α-induced IL8 activation, we found that mBD14 inhibited the expression and protein expression of selected negative regulators of these signaling events (S100A8, TOLLIP, TNFα, TNIP1). In a human immunomodulated keratinocyte cell line (HV-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 TNFα receptor and TNIP1 mRNA expressions significantly, and dose dependently increased in the presence of 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 blot we found a decrease in mBD14 expression 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 pathways. Specialized, endogenous negative regulators do exist in these cells which may be involved in the induced molecular events, and thus can be important for the maintenance of epidermal homeostasis.

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