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IMMUNOPATHOGENESIS OF PSORIASIS: ANTIMICROBIAL PEPTIDES TAKE CENTRE STAGE

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SUMMARY

Psoriasis is a chronic-relapsing inflammatory skin disease characterized by the aberrant activation of dendritic cells that stimulate pathogenic Th17 cells. Two main unsolved issues in the field are the nature of the initial trigger factor for the pathogenic Th17 cascade and the nature of the auto-antigen recognized by these Th17 cells. We found that plasmacytoid dendritic cells (PDC) and their activation to produce type I IFN are critical initial events that drive the development of Th17-mediated inflammation in psoriasis. Activation of PDC is driven by antimicrobial peptides such as LL37, hBD2, hBD3 and Lysozyme, which are overexpressed in psoriatic skin and which can trigger activation of TLR7 and TLR9 in PDC by forming complexes with self-nucleic acids released into the extracellular environment during cell turnover. In addition, we found that the anti-microbial peptide LL37 represents a direct auto-antigen recognized by pathogenic Th17 cells in psoriasis. Thus antimicrobial peptides are on one hand the innate trigger of dendritic cell activation, and, on the other hand, represent the antigenic stimulus and target for pathogenic Th17 cells in psoriasis.

PSORIASIS: A CHRONIC INFLAMMATORY DISEASE OF THE SKIN MEDIATED BY TH17 CELLS

Psoriasis is a common chronic inflammatory skin disease that affects 2 to 3% of the worldwide population (*Lowes* et al., 2007; *Griffiths* and *Barker*, 2007; *Nestle* et al., 2009). In its most prevalent form plaque psoriasis manifests as scaly erythematous plaques that may cover large body areas (Figure 1). Over the past years it has become clear that plaque psoriasis is mediated by T cells producing high levels of Th17 cytokines (*Zheng* et al., 2006; *Zaba* et al., 2007; *Lowes* et al., 2008). The pathogenic Th17 cells are stimulated in the dermis by aberrantly activated conventional dendritic cells producing TNF-α and IL-23 and subsequently migrate into the epidermis where they recognize a yet unknown auto-antigen. As a consequence, pathogenic Th17 cells produce IL-17 and IL-22, which are directly responsible for the keratinocyte hyperproliferation and the development of the psoriasis plaque. The pathogenic role of the Th17 cells in psoriasis is now validated by mouse model of psoriasis, the efficacy of targeting IL-23 or IL-17, and the discovery of genetic polymorphism in the IL-23A and IL-23R genes associated with the development of psoriasis (*Zheng* et al., 2006; *Zaba* et al., 2007; *Lowes* et al., 2008).

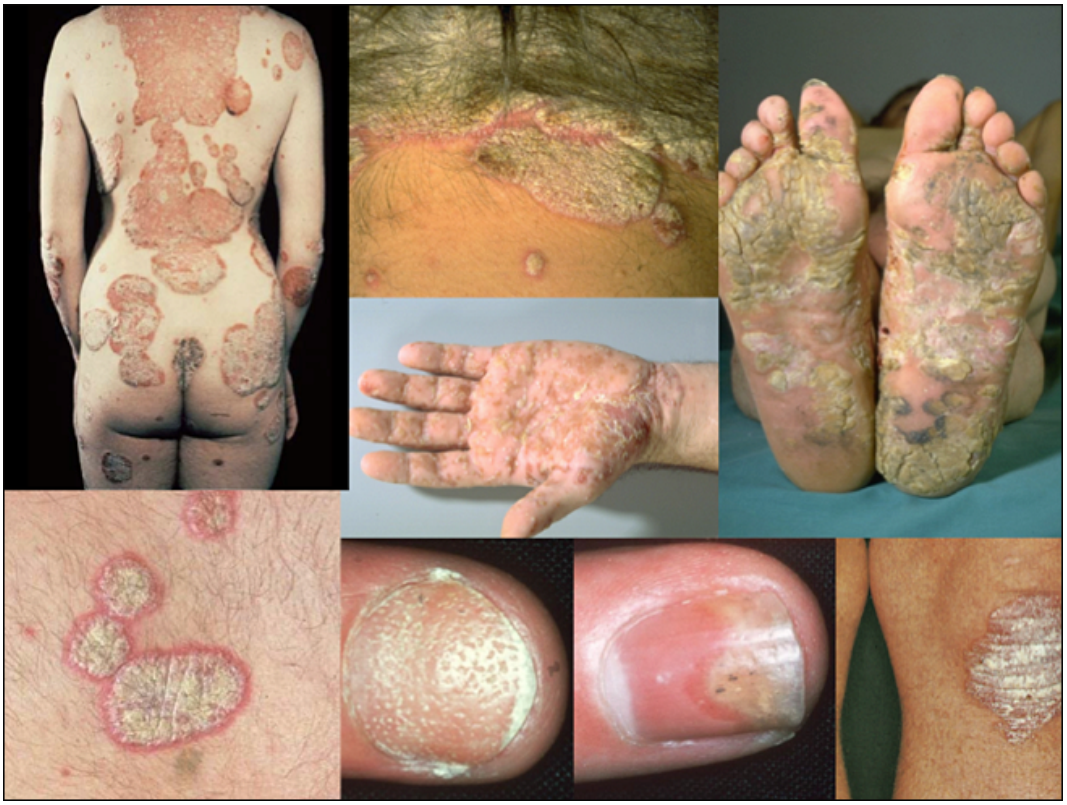

Figure 1: Skin manifestations of psoriasis.

ROLE OF PLASMACYTOID DENDRITIC CELLS AND TYPE 1 INTERFERONS IN THE INITIATION OF PSORIASIS

The role of innate events initiating the pathogenic Th17 cell cascade in the psoriatic skin has been poorly investigated. In 2003 we made an interesting clinical observation in a patient treated topically with the TLR7 agonist imiquimod (Aldara™) for what was thought to be a bowenoid keratosis. After 10 weeks of treatment, the patient showed an enlargement of the lesion along with surrounding satellite lesions consistent with the development of a psoriasis plaque (*Gilliet* et al., 2004). Toll-like receptor 7 (TLR7) is an endosomal receptor for viral single-stranded RNA that is specifically expressed by a subset of human dendritic cells called plasmacytoid dendritic cells (PDC) (*Gilliet* et al., 2008). PDCs are key effectors in antiviral immunity because they express TLR7 along with TLR9, a specific receptor for viral DNA. Upon viral infection, PDC expressing TLR7 and TLR9 sense viral RNA and DNA when brought into the endosomal compartment during the process of infection (*Gilliet* et al., 2008). In response to TLR7 and TLR9 activation, PDC's produce large amounts of type-I IFN (approximately 100 fold than any other cell type of the human body). Type-I IFN produced by PDC provide cell resistance to viral infection but also critically shape antiviral immune responses by maturing conventional dendritic cells expanding memory T cells and

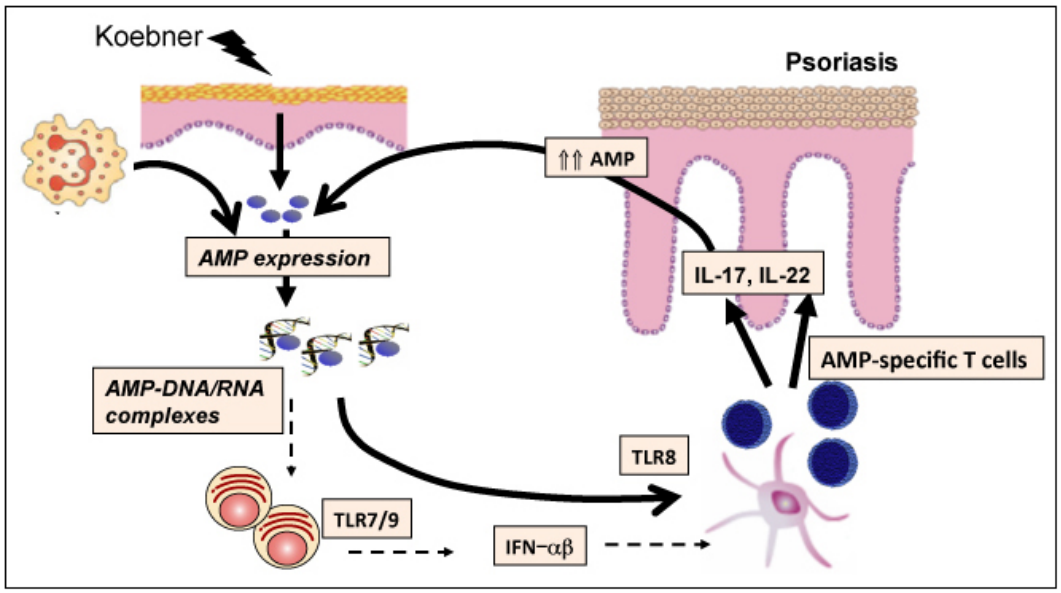

Figure 2: Immunopathogenesis of psoriasis.

activating cytotoxic NK cells (*Gilliet* et al., 2008). Whereas PDC are absent in healthy skin under homeostatic conditions, large numbers of PDC infiltrating the dermis were found in imiquimod treated skin (*Gilliet* et al., 2004) and in developing psoriatic skin lesions (*Nestle* et al., 2005). Not only were these PDC accumulating in developing psoriatic skin lesions, but they were also found to produce large amount of type-I IFN (*Nestle* et al., 2005). The role of PDC and type-I IFN's in the pathogenesis of psoriasis was assessed in a xenotransplant model of human psoriasis that is based on transplantation of un-involved skin of a psoriatic patient on an immunosuppressed (AGR 129) mouse. In this model, the engrafted human skin developes spontaneously into a fully-fledged psoriatic plaque within 35 days upon transplantation, a process mediated by resident Th17 cells. Injection of either neutralizing anti-IFNAR (type I IFN receptor) antibodies or an anti-BDCA2 antibody, which targets specifically human PDC's and blocks their ability to produce type-I IFN, completely inhibited the Th17 cell-dependent development of psoriasis (*Nestle* et al., 2005), indicating that PDC and their activation to produce type-I IFN is an upstream event in the immunopathogenesis of psoriasis (Figure 2).

ANTIMICROBIAL PEPTIDES DRIVE THE ACTIVATION OF PLASMACYTOID DENDRITIC CELLS TO PRODUCE TYPE-I INTERFERONS IN PSORIASIS

But how are PDC's activated to produced type-I IFN in psoriasis, a chronic inflammatory disease not linked to viral infection? To address this question we used fractions derived from HPLC of psoriatic scales to activate

PDC's isolated from peripheral blood. These experiments, followed by extensive biochemical characterization of the IFN inducing fractions using mass spectrometry and sequencing, allowed us to identify lL-37, human β-defensin 3, human β-defensin 2 and lysozyme as activators of PDC's (*Lande* et al., 2007, 2014a). These PDC activators are all cationic antimicrobial peptides, endogenous antibiotics that are normally not expressed in normal skin but produced by keratinocytes or released by infiltrating neutrophils in injured skin. A common feature of these antimicrobial peptides is their cationic and amphiphatic structure, which allows them to associate with bacterial membranes and to form pores in them, thus allowing killing of the microbe (*Zasloff*, 2002). In psoriasis LL-37, hBD2 and hBD3, as well as lysozyme are overexpressed throughout all epidermal layers but some staining can be found in the dermal compartment, where PDC's are located (*Lande* et al., 2007, 2014a), suggesting their role in the activation of PDC's and in the triggering of psoriasis. But how can antimicrobial peptides activate PDC's? PDC's are activated by viral RNA and DNA brought into TLR7 and TLR9 containing endosomal compartments during the infectious process. By contrast, self-RNA and self-DNA released in the context of cell turn-over are unable to activate PDC's because these nucleic acids are rapidly degrading in the extracellular environment and therefore fail to be internalized by PDC's. We found that, via their positive charges, antimicrobial peptides can form complexes with extracellular self-DNA and self-RNA fragments and protect them from extracellular degradation (*Lande* et al., 2007, 2014a; *Ganguly*, 2009). These complexes acquire net positive charges, which allows them to associate with anionic proteoglycans in the membrane of PDC's. As a consequence the nucleic acid complexes are endocytosed reaching intracellular TLR7 and TLR9 compartments, where they trigger the production of type I IFN's. Thus, cationic antimicrobial peptides IL-37, hBD2, hBD3, and lysozyme can break innate tolerance to self nucleic acids and lead to innate immune activation of PDC's via TLR7 and TLR9. Interestingly, we also found that RNA-AMP complexes can directly activate conventional DC's via TLR8 (*Ganguly*, 2009). Based on these findings, we propose the following model: antimicrobial peptides, induced during mechanical stress of the skin (Koebner phenomenon) form complexes with extracellular self-nucleic acids. These complexes trigger activation of TLR7 and TLR9 in plasmacytoid dendritic cells to induce type-I IFN and/or TLR8-expressing conventional dendritic cells that directly stimulate activation of pathogenic Th17 cells (Figure 2).

OVEREXPRESSION OF ANTIMICROBIAL PEPTIDES DRIVES SUSTAINED PLASMACYTOID DENDRITIC CELL ACTIVATION, LEADING TO PSORIASIS

In healthy individuals, skin injury is linked to a well-controlled and transient activation of PDC's by AMP-nucleic acid complexes, which promotes short-term inflammation and wound re-epithelialization (*Gregorio* et al., 2010). In psoriasis patients, skin injury leads to an exaggerated and persistent activation of PDC's due to an overexpression of AMP's. This leads to chronic inflammation, which ultimately drives epidermal hyperproliferation and develop-

ment of the psoriatic plaque (*Lande* et al., 2010). But what drives the constant overexpression of antimicrobial peptides in psoriasis? One important factor is the high levels of Th17 cytokines in the plaque, which contribute to the constant activation of keratinocytes to produce antimicrobial peptides. Indeed both IL-17 and IL-22 alone or in combination have been shown to trigger expression of antimicrobial peptides in keratinocytes (*Wolk* et al., 2004; *Liang* et al., 2006). Another interesting element is the identification of human β-defensin copy number polymorphism associated with the development of psoriasis, providing a genetic basis for the AMP overexpression in psoriasis (*Hollox* et al., 2008).

ANTIMICROBIAL PEPTIDES AS AUTO-ANTIGENS RECOGNIZED BY PSORIATIC T-CELLS

Because antimicrobial peptides are taken up by dendritic cell subsets we asked ourself whether these antimicrobial peptides could serve as auto-antigens and are presented to auto-immune T-cells in the psoriatic plaque. To address this question we used peripheral blood mononuclear cells from 52 psoriatic patients and stimulated them with either LL-37 or a scrambled form of the LL37 peptide. We found in approximately 40% of the patients that LL-37 induced a T-cell proliferation which was not present in control populations including healthy donors, scleroderma patients, erysipelas patients and atopic dermatitis patients (*Lande* et al., 2014b). LL-37 reactive T-cells did not only proliferate, but did also produce IFN-γ, and TH17 cytokines, IL-17 and IL-22 (*Lande* et al., 2014b). LL-37 reactive T-cells were both of CD4 and CD8 phenotype. Several CD4 and CD8 T-cell lines and clones were obtained and MHC restriction was demonstrated. Interesting, HLA-Cw6, that is found in 50% of psoriasis patients and is highly associated with the development of psoriatic disease, was found to be an excellent binder of LL-37. We also generated tetramers, which were able to detect LL-37 specific T-cells in the circulation of psoriasis patients (*Lande* et al., 2014b). A significant correlation between the presence of circulating IL-37 specific T-cells and the disease activity was observed. In addition, about 80% of patients with severe psoriasis (PASI >10) displayed circulating IL-37 specific T-cells. These findings alone with the fact that LL-37 specific T-cells produce pathogenic TH17 cytokines and are present in skin lesions suggests that LL-37 specific T-cells may be pathogenic T-cells in psoriasis. Accordingly, patients undergoing disease remission during anti-TNF treatment displayed decreased proliferative activity and tetramer staining of their LL37-specific T cells. Furthermore, LL37-specific T cells lost skin homing receptors CCR10, CLA, CCR6, and their ability to produce IL-17 and IL-22 (*Lande* et al., 2014b). More recent *in vivo* mouse studies, based on the repetitive injection of antimicrobial peptides into mouse skin, demonstrated a direct pathogenic role of AMP-specific T-cells. The injection of AMP induced the expansion of AMP-specific T cells in the skin and the development of a psoriatic phenotype, which was entirely T cell dependent.

CONCLUSION AND OPEN QUESTIONS

Cationic antimicrobial peptides are endogenous antibiotics that are expressed in injured skin. In addition to providing protection against microbial invasion we identified a unique pro-inflammatory function of these peptides through their ability to transport self-nucleic acids into intracellular compartment and promote activation of nucleic acid-recognizing Toll-like receptors. In psoriasis, cationic antimicrobial peptides LL37, hBD2, hBD3 and lysozyme are overexpressed by keratinoytes and drive a constant activation of dendritic cells including plasmacytoid dendritic cells producing type-I IFN and conventional dendritic cells producing IL-23 and TNF (Figure 2). On the other hand antimicrobial peptides may also represent auto-antigens presented by the activated dendritic cell subsets to autoimmune T cells, leading to their activation and expansion (Figure 2). Activated antimicrobial peptide-specific T cells may then migrate into the epidermis where they recognize the antimicrobial peptide expressed by keratinocytes and produce IL-17, IL-22 leading to the typical epidermal hyperproliferation that drives psoriasis (Figure 2).

A number of key questions remain to be answered. First, what is the structural requirement of antimicrobial peptide to be immunogenic and are all the antimicrobial peptides that drive activation of dendritic cells also T cell autoantigen? Second, do antimicrobial peptide-specific T cells escape central tolerance, or are these low-affinity T cells that are stimulated in the periphery in the context of a strong innate activation of dendritic cells? Third, what are the exact mechanisms underlying the concerted overexpression of several antimicrobial peptides in psoriasis? Is there a role for the microbiome? Finally, how can we target a broad range of antimicrobial peptides to block their immunogenicity for the treatment of psoriasis?

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