Superficial Immunity: Antimicrobial Responses Are More Than Skin Deep

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The skin barrier is essential for host defense, but how the skin provides protection when the barrier is breached is not well understood. In this issue of *Immunity*, Gallo and colleagues report that keratinocytes integrate signals from antimicrobial peptides via MAVS signaling to amplify their antiviral immune response.

As the largest and most exposed barrier surface, the skin provides the first line of defense against pathogens such as bacteria and viruses. A growing body of literature has revealed that the skin possesses unique immune properties to optimize host-protective immunity. For example, antimicrobial peptides (AMPs) such as LL37 are produced by keratinocytes to mediate rapid antibacterial responses in the setting of skin barrier breach. In addition to their bactericidal functions, AMPs are also known to play a regulatory role in other immune processes. In this issue of *Immunity*, Zhang et al. (2016) demonstrate that LL37 amplifies keratinocyte-intrinsic interferon (IFN)-β-mediated antiviral programs in response to wounding. While this response is critical to prevent bacterial and viral infection, dysregulation of the keratinocyte-LL37-IFN-β axis can lead to autoimmune diseases such as the inflammatory skin disorder psoriasis.

Upon detection of viral nucleic acid, infected cells synthesize and release type I IFNs, typically IFN-α and IFN-β, which activate antiviral programs within infected as well as neighboring stromal cells. This response directly controls the spread of infection within the stroma but also recruits immune cells to promote viral clearance. Zhang et al. employ immunofluorescence imaging of skin biopsies and 3D organotypic human skin cultures to show that keratinocytes produce IFN-β in response to skin wounding and double-stranded RNA (dsRNA) exposure. While previous work has shown that human keratinocytes are capable of producing IFN-β in response to dsRNA (Fujisawa et al., 1997), the current study provides evidence for robust IFN-β production by human keratinocytes in response to biopsy-induced wounding. Unexpectedly, this antiviral program appears to be regulated by the antibacterial peptide LL37, which directly enhances the IFN-β response.

To identify how LL37 modulates this keratinocyte-intrinsic antiviral activity in response to wounding, Zhang et al. examined signaling pathways known to be activated by dsRNA. Extracellular dsRNA can be detected in the endosome through TLR3 or directly in the cytosol by the RIG-I-like receptors (RLRs). The authors have previously shown that dsRNA binds TLR3 on keratinocytes to modulate their function (Lai et al., 2009). However, in this study, they found that LL37-mediated production of IFN-β was dependent upon mitochondrial antiviral signaling (MAVS) protein, a critical component of RLR signaling pathways (Chen et al., 2007). Thus, it appears that LL37 modifies dsRNA detection from damaged cells by promoting distinct intracellular pathways leading to the type I IFN response (Figure 1). Specifically, LL37 directly binds and represses an inhibitory isoform of TANK-binding protein kinase 1 (TBK1) downstream of the MAVS pathway. This inhibitory isoform of TBK1, called TBK1s, is a constitutive inhibitor of IFN-β production. Thus, LL37 permits signaling through the MAVS pathway, which is normally dampened under homeostatic conditions. Collectively, these findings reveal a previously unrecognized pathway by which keratinocyte-specific IFN-β responses are regulated by a canonical antimicrobial peptide.

In contrast to their protective role in host defense, overproduction of type I IFNs has been implicated in a number of autoimmune disorders including systemic lupus erythematosus (SLE) and psoriasis. For example, IFN-α produced by plasmacytoid dendritic cells (pDCs) in the dermis is an important early driver of psoriasis development, and blocking antibodies against the type I IFN receptor (IFNAR) halt progression of psoriatic skin lesions in mouse xenografts (Nestle et al., 2005). Additionally, dysregulated IFN-β responses have been associated with the development of psoriasis. Zhang et al. find that IFN-β is highly upregulated in the epithelium of psoriasis lesions compared to non-lesional and healthy control skin using immunofluorescence microscopy. In support of this, a recent translational study identified that a variety of interferon-stimulated genes such as *ISG15*, *MX1*, and *OAS2* are markedly elevated in the setting of psoriasis in patients (Wolk et al., 2013). However, in this previous study, the primary mechanism by which these responses were regulated was through IL-29 derived from T helper type 17 (Th17) cells. Thus, it appears that there are multiple regulatory pathways by which both epithelial and immune cells promote type I IFN responses in psoriasis.

By examining how IFN-β can contribute to psoriasis pathogenesis, Zhang et al. found that conventional DCs (cDCs) mature and induce T cell proliferation

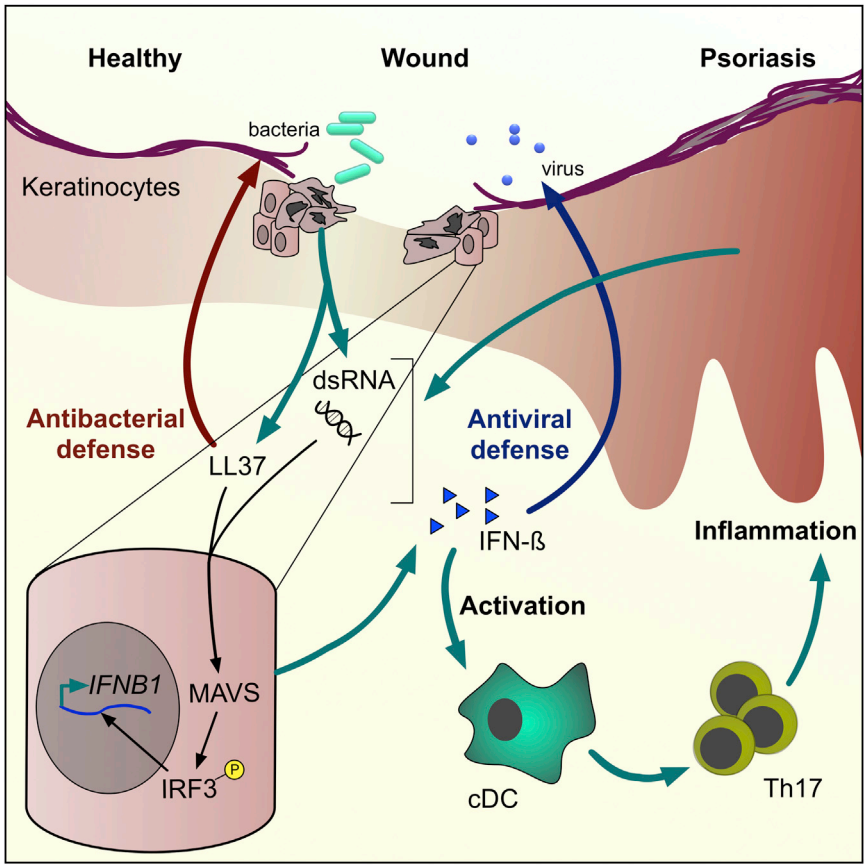

Figure 1. Keratinocytes Amplify IFN-β Production in Response to LL37 and dsRNA Released from Damaged Skin

LL37 and dsRNA are released from damaged keratinocytes during wounding and in psoriasis lesions. dsRNA is detected by cytosolic sensors, which together with LL37 signal through MAVS to produce phosphorylated IRF3. Phosphorylated IRF3 can then initiate transcription of *IFNB1* in keratinocytes, which in turn activates cDCs to promote inflammation. In psoriasis, cDCs recruit Th17 cells to drive disease pathogenesis. In addition to the role of LL37 in mediating direct antibacterial defense, it provides antiviral activity via regulation of IFN-β. IFN, interferon; dsRNA, double-stranded RNA; MAVS, mitochondrial antiviral signaling protein; IRF3, interferon regulatory factor 3; cDC, conventional dendritic cell; Th17, T helper type 17.

when exposed to keratinocyte-derived IFN-β. Thus, while pDCs are a critical source of IFN-α that acts directly on keratinocytes to mediate skin pathology, IFN-β might act indirectly by licensing cDCs to activate effector T cell responses (Figure 1). Despite their shared use of the IFNα/β receptor complex and redundant signaling pathways, these data provoke the hypothesis that different type I IFN species might have distinct immunological functions. In support of this, a recent study of lymphocytic choriomeningitis virus (LCMV) infection suggests that IFN-α might be more important for prevention of early viral dissemination, whereas IFN-β is critical for adaptive immunity and viral clearance (Ng et al., 2015). However, these specialized functions might become dysregulated in the setting of autoimmunity and can result in diseases such as psoriasis.

Strikingly, despite the known redundancy of IFN-α and IFN-β, Zhang et al. demonstrate that there is very little anatomic and cellular overlap in terms of their expression pattern. Indeed, in both wounding and psoriasis, pDCs exclusively expressed IFN-α, while keratinocytes were the clear source of IFN-β. These findings provoke the hypothesis that spatial segregation of type I IFN expression might result in specialized immune responses. In support of this broad concept, studies examining antigen-presenting cells (APCs) in the skin have revealed that similar spatial segregation allows for highly specific immune responses within different anatomic regions of the skin. In response to *Candida* infection, Langerhans cells (LCs) located in the epidermis selectively promote T helper type 17 (Th17) responses, while a population of dermal DCs favor T helper type 1 (Th1) and cytotoxic T cell responses (Igyártó et al., 2011). Collectively, these findings suggest that distinct anatomic localization allows for optimal responses in the skin to heterogeneous populations of opportunistic pathogens. However, the significance of this spatial diversity remains to be determined in the setting of type I IFN biology.

Extending beyond psoriasis, these findings shed light on another common inflammatory skin disorder atopic dermatitis (AD), also known as eczema. In contrast to psoriasis, which is associated with elevated expression of AMPs including LL37, AD patients exhibit deficiency of these factors in the involved skin. Thus, they markedly differ in terms of their responses to opportunistic pathogens. Whereas psoriasis patients do not typically suffer from chronic bacterial or viral infections, AD is frequently complicated by recurrent superinfections with bacteria, especially *Staphylococcal* and *Streptococcal* species. Severe AD patients also suffer from widespread viral infections, including herpes simplex virus infection, which manifests as the complication known as eczema herpeticum. Although AMP deficiency is known to result in susceptibility to bacteria in AD, why skin antiviral immunity is impaired in these patients is not well understood. The findings by Zhang et al. show how LL37 deficiency could lead to not only impaired antibacterial responses but also subsequent loss of antiviral immunity. In support of this, a recent study found that AD patients exhibited diminished levels of antiviral proteins known to be induced by type I IFNs (Wolk et al., 2013).

Epithelial barrier surfaces have evolved conserved mechanisms by which they mediate innate host defense through factors such as AMPs that act directly on pathogens independently of effector immune cells. However, Zhang et al. identify a novel mechanism by which the AMP LL37 enables keratinocytes to mount an immune response through the production of IFN-β. This dual role of LL37 in host defense allows for

advantageous efficiency by coupling antibacterial and antiviral immunity. However, this might represent a double-edged sword as dysregulation of this mechanism can lead to autoimmune inflammation and psoriasis. Thus, while LL37 is classically thought to act mainly on surface pathogens, its true functions might be more than skin deep.

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Innate B Cells Tell ILC How It's Done

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Innate lymphoid cells (ILCs) are known as first responders to infections and as instructors of subsequent CD4$^+$ T cell cytokine profiles. In this issue of *Immunity*, Fan and colleagues now demonstrate that even earlier responding innate-like B cells (NKB) induce these protective ILC responses.

The recent discovery of three distinct groups of the innate lymphoid cells (ILCs) 1, ILC2, and ILC3, which closely mirror in transcription factor expression and cytokine profile that of the CD4$^+$ T cell effectors, Th1 (T-bet and IFN-γ), Th2 (GATA-3 and IL-4), and Th17 (RORγt and IL-17 and IL-22), respectively, has cemented the view that CD4$^+$ T cells and their innate "look-alikes" control the quality of the induced response via the cytokines they produce (Bando and Colonna, 2016). Although numerous studies have pointed to important regulatory roles of cytokine producing B cells in the orchestration of immunity (Lund and Randall, 2010), overall their contributions have received little attention. A study described in this issue of *Immunity* by Fan and colleagues (Wang et al., 2016) is likely to change this perception and have B cell immunologists walk just a little taller. The study shows that innate-like B cells, through their production of interleukin-12 (IL-12) and IL-18, are in the "driver's seat" of protective immune responses to intracellular pathogens, regulating interferon-γ (IFN-γ) production by NK cells and ILC1.

IFN-γ production by innate lymphocytes is critical for the early control of numerous viral and intracellular bacterial infections. IFN-γ induces the production of microbicidal reactive oxygen species in myeloid cells, regulates production of antibody classes that enhances antibody-mediated cytotoxicity, enhances antigen presentation by antigen presenting cells (APC), and through a positive feedback loop further promotes CD4$^+$ Th1 cell differentiation (Shtrichman and Samuel, 2001). Thus, the early ILC1 responses instruct the induction of Th1 cell responses, perpetuating a protective IFN-γ dependent adaptive immune response (Bando and Colonna, 2016). Although it is known that ILC1 and NK cells can respond to IL-12, IL-15, and IL-18 cytokines with IFN-γ production, it has been thought that myeloid or epithelia cells provide these signals and/or that these cells have an inherent ability to rapidly produce IFN-γ in response to microbial challenges, thus suggesting that they are the first lymphocyte responders, controlling the quality of the induced immune response.

These views are now brought into question by Wang et al. (2016), who demonstrate that NK and ILC1 are not instructed by cytokine-producing epithelial or myeloid cells, neither are they pre-programmed to produce large amounts of IFN-γ. Instead, the production of IL-12 and IL-18 is driving IFN-γ responses by NK cells and ILC1 in response to a variety of viral and microbial infections, including murine cytomegalovirus (MCMV), herpes simplex virus (HSV), *Salmonella Typhimurium*, *Listeria monocytogenes*, and