

The Central Role of Bcl-3 in Atopic Dermatitis

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Cytokines and hormones are essential mediators in the regulation of antimicrobial peptide and protein (AMP) levels during host defense responses. In this issue, Büchau *et al.* identify and characterize B-cell leukemia-3 as an important modulator of AMP expression during the innate immune response in keratinocytes as well as in the chronic inflammatory skin disorder atopic dermatitis.

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Antimicrobial peptides and proteins

Humans are constantly exposed to infection by pathogenic microorganisms and agents. In most cases, we are able to resist infection with the help of immunity, which is composed of two major defenses: innate or nonspecific immunity, which acts immediately after infection, and adaptive or specific immunity, which is more powerful and pathogen specific. When skin is breached by an injury, pathogens that invade the host are initially eliminated by mobilized cellular host defenses, including neutrophils, macrophages, mast cells, eosinophils, and natural killer cells. These cells express a variety of recognition receptors, such as toll-like receptors (TLRs), which are activated in part by microbial pathogens. TLRs then promote the release of mediators, including those that initiate the complement cascade, cytokines, chemokines, superoxides, nitric oxides, and antimicrobial peptides and proteins.

Antimicrobial peptides (AMPs) and proteins (including defensins, cathelicidin, histatins, human β -defensin (HBD), cathepsin G, high-mobility group box-1 (HMGB1), and lactoferrin; Kolls *et al.*, 2008)—which are produced by different inflammatory and noninflammatory cells, including keratinocytes—have various mechanisms of action and are divided into

different groups. Many of the antimicrobial peptides are produced constitutively and stored in secretory granules, and others are induced by proinflammatory stimuli such as IL-1, tumor necrosis factor (TNF), and lipopolysaccharide (Kolls *et al.*, 2008). With the exception of HBD1, most AMPs are undetectable in skin under basal conditions but are induced after injury or inflammatory stimuli.

AMPs are believed to act by depolarizing and/or piercing bacterial cell membranes. Besides killing gram-positive and -negative bacteria, AMPs can kill fungi and certain viruses.

In addition to their antimicrobial activity, AMPs can regulate innate immune responses by inducing chemokines and cytokines. Cytokine signaling leads to the recruitment of inflammatory cells, including macrophages and neutrophils, which further increase antimicrobial protein responses (Kolls *et al.*, 2008). However, negative feedback regulation by some AMPs has previously been reported to diminish inflammation. Cytokines and hormones such as vitamin D are well-known mediators in AMP regulation in keratinocytes. Specifically, the vitamin D response element was identified in the promoter region of the human cathelicidin gene (Schauber *et al.*, 2007). Stimulation of keratinocytes with the active metabolite

1,25-(OH)₂ vitamin D3 (1,25D3) increased transcription and activation of cathelicidin, leading to antimicrobial activity against *Staphylococcus aureus* (Schauber *et al.*, 2008).

B-cell leukemia-3 and keratinocytes

A variety of microbial components activate the transcription factor nuclear factor- κ B (NF- κ B), which is important in the activation of host immune responses. The receptor-mediated signaling pathway leading to NF- κ B activation by the microbial components is mainly through TLR ligands and proinflammatory cytokines such as TNF and IL-1. In nonstimulated cells, NF- κ B is located in the cytoplasm; upon activation it translocates into the nucleus, where it activates the transcription of target genes. Five members of the NF- κ B protein family have been discovered: p50, p52, p65, RelB, and c-Rel. B-cell leukemia-3 (Bcl-3), a member of the inhibitor NF- κ B family, will interact only with p50 and p52 (Massoumi and Paus, 2007). The role of Bcl-3 during NF- κ B signaling is complicated and varies depending on the manner of activation and the cell type that responds. In keratinocytes, activation of Bcl-3 by 12-O-tetradecanoylphorbol-13 acetate or UV radiation caused rapid translocation of Bcl-3 to the nucleus and recruitment of p50/p52 to target genes for the transcription of genes such as cyclin D1 (Massoumi *et al.*, 2006). In addition, sustained Bcl-3 nuclear translocation led to the development of skin papillomas (Massoumi *et al.*, 2006) or squamous-cell carcinomas (Budunova *et al.*, 1999), by augmentation of p50/p52-related NF- κ B binding in mouse keratinocytes. In other systems, Bcl-3 can act as a transcription repressor by binding to p50 homodimers. For example, the repressor function of Bcl-3 was recently demonstrated in TLR signaling, where the tolerance and suppression were dependent on the coordinated action of a p50–Bcl-3 complex. In the absence of this complex, loading of NF- κ B subunits on target promoters,

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which is critical for appropriate gene expression, was disrupted, leading to aberrant expression of inflammatory genes. This suggested that inflammatory responses can be controlled effectively by targeting the NF- κ B p50 and Bcl-3 pathway (Carmody *et al.*, 2007).

Blocking Bcl-3 downstream signaling is a potential therapy in AD.

Even though the means by which AMP expression is regulated by different cytokines during infection and inflammation has been established, it is not known which targets can respond to the related cytokine profile and thereby affect AMP expression directly. Büchau *et al.* (2009, this issue) identify Bcl-3 as an important modulator for expression of AMPs during innate responses by keratinocytes. Indeed, two potent mediators of the Th2-driven innate response, IL-4 and

IL-13, induced Bcl-3 expression. This, in turn, led to the downregulation of HBD3 expression. Further, when Bcl-3 expression in keratinocytes was silenced by siRNA, the downregulatory effect of IL-4 on that expression was reversed. In contrast, TNF- α -mediated upregulation of HBD3 via Th1 cytokines IL-6 and IL-8 was reduced in the presence of Bcl-3 siRNA (Figure 1). Together, these results highlight Bcl-3 as a common effector molecule for both Th1 and Th2 cytokines in regulating AMP levels during the innate responses by keratinocytes.

Atopic dermatitis

Atopic dermatitis (AD) is a chronic inflammatory skin disorder that causes patients to have frequent bacterial and viral skin infections, which can eventually advance to superinfection. Although the cause of AD is not entirely clear, its etiology involves a complex interplay among environmental triggers and genetic factors, including altered innate and adaptive immune responses. Further, AD patients' defective innate immune systems may allow bacterial infections to persist despite therapy (Wollenberg and Bieber, 2000;

Leung and Bieber, 2003). The patients' susceptibility to pathogens and infections has been related to diminished recruitment of innate immune cells to the skin, epithelial barrier disruption, TLR2 defects, and a reduction in AMPs (Dokmeci and Herrick, 2008). The reduction in AMP levels in these patients was correlated with the overexpression of Th2 cytokines such as IL-4 and IL-13. Because IL-4 induces IgE production in B cells, an eventual suppression of anti-infectious immune responses will occur via the reduction of AMPs and inhibition of Th1 immunity. Other studies demonstrate that IL-8, which is a potent chemokine that attracts polymorphonuclear leukocytes (PMNs) into the skin where they phagocytize and kill bacteria, upregulates the expression of HBD2 and HBD3. This picture became even more certain when it was shown that TNF- α -induced IL-8 expression was inhibited by IL-4, demonstrating that interplay between cytokine profiles can regulate the expression of AMPs (Bieber, 2008).

The report by Büchau *et al.* (2009) is the first study to connect the function of Bcl-3 to AD and explain how Bcl-3 can decrease the expression of AMPs mediated by the related cytokine profile observed in AD patients. Initial observations indicate that Bcl-3 is overexpressed at both the mRNA and protein levels in lesional skin from AD patients compared with normal skin. Because Th2 cytokines such as IL-4 and IL-13 are overexpressed in AD patients, normal human keratinocytes were exposed to these cytokines. Interestingly, a combination of IL-4 and IL-13 enhanced the levels of Bcl-3 in keratinocytes in the cytoplasm, suggesting that elevated Bcl-3 expression in AD is directly controlled by the levels of IL-4 and IL-13 (Büchau *et al.*, 2009).

Earlier studies documented the role of vitamin D in the expression of the cathelicidin gene via stimulation of keratinocytes with the active metabolite 1,25D₃, in which an increase in cathelicidin levels was observed (Schauber *et al.*, 2007). To determine whether vitamin D can regulate Bcl-3 levels, AD patients were treated

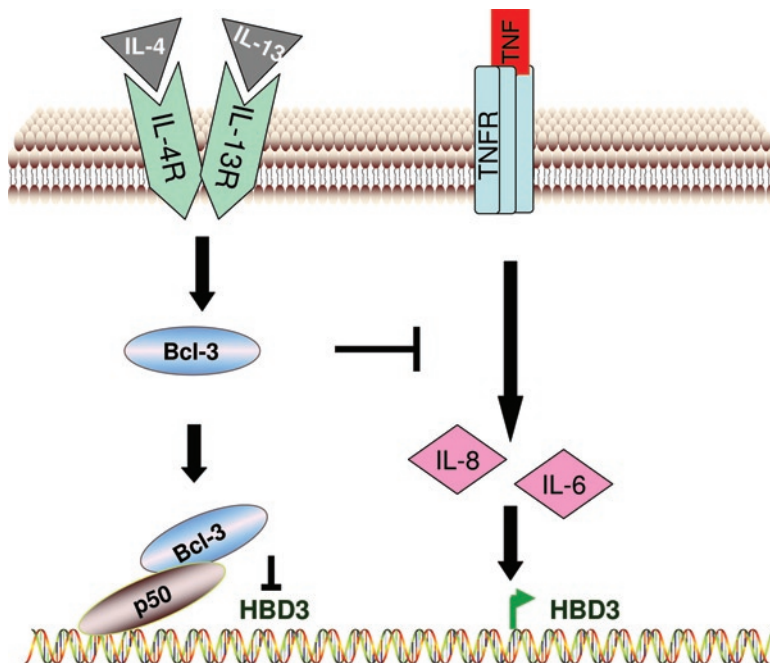


Figure 1. Downregulation of human β -defensin-3 (HBD3) levels by B-cell leukemia-3 (Bcl-3). Increased expression of Bcl-3 in keratinocytes by IL-4 and IL-13 causes downregulation of HBD3 levels. The effect of TNF- α -mediated production of IL-6 and IL-8, as well as HBD3 expression levels, was blocked in the presence of Bcl-3. TNFR, tumor necrosis factor receptor.

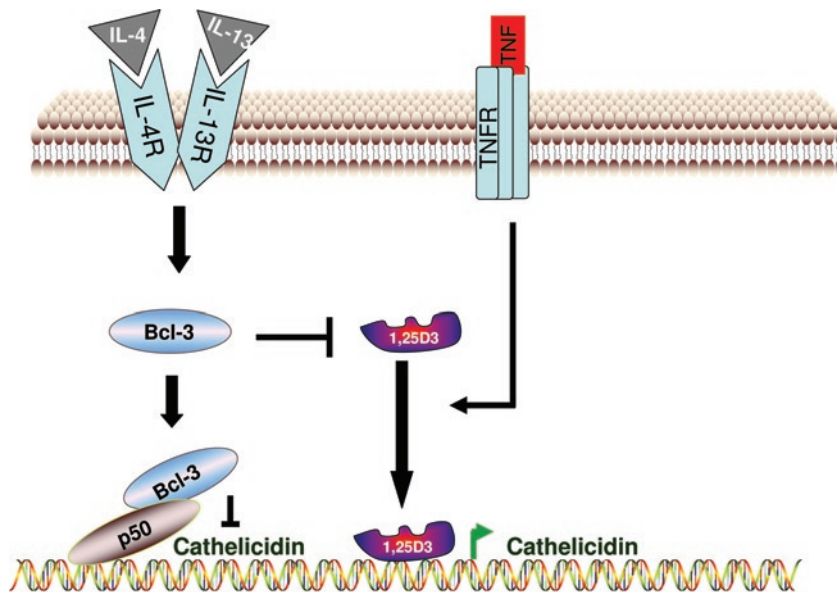


Figure 2. Downregulation of cathelicidin levels by B-cell leukemia-3 (Bcl-3). Tumor necrosis factor- α (TNF- α) can induce cathelicidin only in the presence of 1,25-dihydroxyvitamin D3 (1,25D3). Increased expression of cathelicidin in keratinocytes by 1,25D3 and TNF- α is inhibited in the presence of Bcl-3. TNFR, tumor necrosis factor receptor.

with orally administered vitamin D, resulting in downregulation of Bcl-3 expression in lesional AD skin in a majority of subjects (Büchau *et al.*, 2009). To further confirm that Bcl-3 signaling is important in the regulation of cathelicidin levels, keratinocytes were treated with siRNA against Bcl-3 before stimulation with 1,25D3. 1,25D3 strongly induced cathelicidin gene expression; this effect was further enhanced when the levels of Bcl-3 were reduced (in the presence of Bcl-3-siRNA; Figure 2). To determine whether the Bcl-3-p50 complex is important for this pathway, Büchau *et al.* (2009) used a blocking peptide against p50 and found an effect similar to that of Bcl-3 silencing. This indicated that the regulation of cathelicidin expression involves blocking of p50 subunits by Bcl-3 in the promoter region of human cathelicidin.

Future directions

The elegant study performed by Büchau *et al.* (2009) can be seen as a major step toward clinical trials for AD treatment, but there are still questions that must be addressed. Is the effect of vitamin D or IL-4/IL-13 on human Bcl-3 levels direct or indirect? Can the Bcl-3-p52 complex share the

same target gene and repressor activity in AD as Bcl-3-p50? What percentage of nuclear localized Bcl-3 in AD and keratinocytes is stimulated by Th2 cytokines? Are Bcl-3 levels dependent entirely on promoter activation or can posttranslational modification of Bcl-3, including ubiquitination, play a role in AD? Is the level of CYLD altered in these patients?

It has previously been shown that inactive Bcl-3 is localized mainly in the cytoplasm, whereas the active form of Bcl-3 translocates into the nucleus for binding to the promoter of different genes. Furthermore, Bcl-3 levels can be regulated by polyubiquitination. Therefore, the fate of Bcl-3 is decided by the type of ubiquitin chains attached. Bcl-3 has been shown to be a substrate for both lysine 63 and lysine 48 ubiquitin chains. Lysine 63 mediates nuclear localization of Bcl-3, whereas lysine 48 directs Bcl-3 to the proteasome for degradation. An increase in Bcl-3-lysine 63 ubiquitination, which might be detected in AD patients, can mediate stabilization as well as sustained downstream activity/signaling of Bcl-3. A loss of the *CYLD* gene, which blocks activity of Bcl-3 signaling, could be another possible explanation for the role of Bcl-3 in AD patients.

Taking together these findings, Bcl-3, which was previously shown to act as an oncogene in skin-mediated tumorigenesis and melanoma, seems to have an essential role in AD; therefore, blocking Bcl-3 downstream signaling in keratinocytes may be a potential therapeutic target in treating AD.

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CONFLICT OF INTEREST

The author states no conflict of interest.

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