

Bcl-3 puts the brakes on contact hypersensitivity

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B-cell lymphoma (Bcl)-3 is a nonclassical member of the I κ B protein family known to interact with transcriptionally inactive NF- κ B1 and NF- κ B2 homodimers to modulate gene expression. Besides its action as an oncoprotein, Bcl-3 has been shown to have both proinflammatory and anti-inflammatory functions depending on the cell-type affected. In this issue of the *European Journal of Immunology*, Tassi et al. [Eur. J. Immunol. 2015. 45: 1059–1068] report that Bcl-3 inhibits the production of the proinflammatory chemokines CXCL9 and CXCL10 in keratinocytes, thereby restricting the influx of CD8⁺ effector T cells in a mouse model of allergic contact dermatitis. In addition, mice with a global deficiency of Bcl-3 show enhanced ear swelling responses in the late phase of contact hypersensitivity responses. Besides keratinocytes, other radioresistant cell types appear to also utilize Bcl-3 to dampen the inflammatory response. This Commentary will discuss the evidence supporting Bcl-3 as a critical player in limiting inflammation during the later stages of contact hypersensitivity.

Keywords: Bcl-3 · CXCL9 · CXCL10 · Contact hypersensitivity · Keratinocytes · NF- κ B



See accompanying article by Tassi et al.

Allergic contact dermatitis is a common form of skin allergy that affects 5–10% of the population in western countries, often as a result of occupational exposure to contact sensitizers, such as metal ions or small reactive chemicals contained in cosmetics, fragrances, or paints [1]. Because of their small size, contact sensitizers easily penetrate the skin and, as reactive haptens, covalently bind and modify self-proteins. Such modified self-peptides are processed by skin-resident DCs and induce adaptive T-cell responses [1]. More recently, contact sensitizers have been shown to act as adjuvants themselves, initiating innate immune reactions through the stimulation of TLRs and activation of the inflammasome [2, 3]. In mice, allergic contact dermatitis is usually modeled as a contact hypersensitivity (CHS) reaction, consisting of a sensitization and an elicitation phase.

In the present issue of the *European Journal of Immunology*, Tassi et al. [4] employ the mouse model of CHS to investigate the function of B-cell lymphoma (Bcl)-3, a transcription factor of the I κ B family, in the context of this allergic skin reaction. Bcl-3 is an atypical member of the I κ B family, which includes I κ B α , I κ B β , and I κ B ϵ primarily retain and inhibit NF- κ B in the cytoplasm, and are subjected to inducible degradation to allow for activation and nuclear translocation of NF- κ B factors [6]. In contrast, Bcl-3 binds to p50/NF- κ B1 or p52/NF- κ B2 homodimers and renders them transcriptionally active, resulting in either a transactivating or an inhibitory complex, depending on the cellular context and the relevant target gene [5]. Likewise, Bcl-3 was shown to interact with histone acetyl transferases as well as histone deacetylases [5]. Because of this functional ambiguity in transcriptional regulation, Bcl-3 has been shown to act in either pro- or anti-inflammatory capacities in different disease models. For example,

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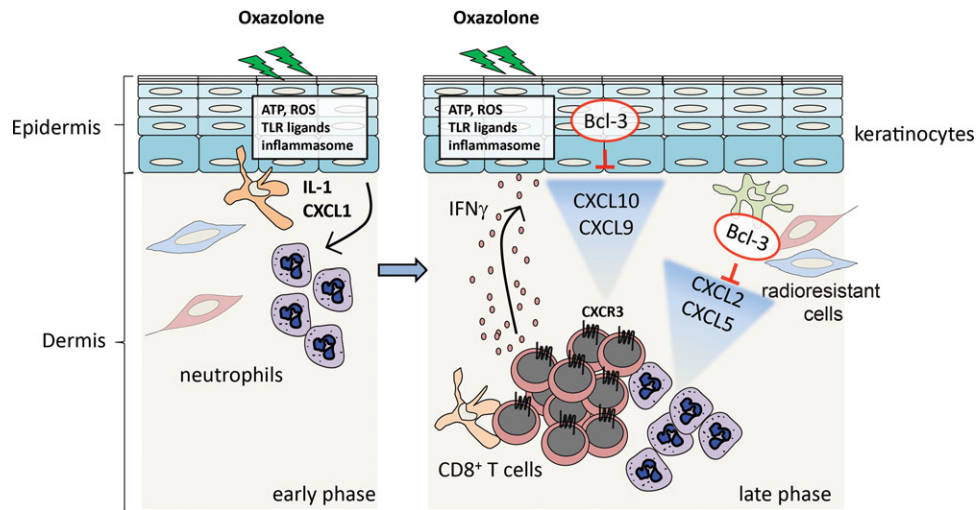


Figure 1. Suppressive role of Bcl-3 in CHS. Contact allergens, such as oxazolone, activate the innate immune system of the skin, leading to the recruitment of neutrophils in the early phase of CHS (left). In the late phase (right), chemokines such as CXCL2, CXCL5, CXCL9, or CXCL10 are produced and attract IFN- γ -producing CD8 $^{+}$ T cells besides neutrophils. IFN- γ activates keratinocytes to enhance production of CXCL9 and CXCL10, thereby amplifying the inflammatory response by recruiting more effector T cells via CXCR3. Expression of Bcl-3 in radioresistant cells was shown by Tassi et al. [4] to block the production of inflammatory chemokines and thus downregulate leukocyte infiltration to the site of CHS induction. In particular, Bcl-3 expression in keratinocytes selectively inhibits the release of CXCL9 and CXCL10 and therefore restricts the inflammatory response.

production of IL-17 and development of autoimmune diabetes was found to be enhanced in Bcl-3-deficient NOD mice [7], whereas Bcl-3 deficiency was shown to protect mice from development of chemical-induced colitis due to enhanced proliferation of intestinal epithelial cells [8].

The Siebenlist laboratory has made seminal contributions to understanding the biological function of Bcl-3 as an oncoprotein [9, 10], in the control of bacterial infection [11], as well as development of immunological tolerance and autoimmunity [12, 13]. In this issue, Tassi et al. [4] analyze the responsiveness of Bcl-3-deficient mice to contact hypersensitivity induced by the contact sensitizers oxazolone or dinitrofluorobenzene. Interestingly, Bcl-3-deficient mice were shown to develop an enhanced and prolonged ear swelling response particularly in the later phase of elicitation, at 48–96 h after chemical challenge, which is known to mainly depend on the influx of CD8 $^{+}$ Tc1 and Tc17 cells (Fig. 1) [1]. At this stage, significantly enhanced numbers of both CD8 $^{+}$ T cells and neutrophils were detected in the inflamed ears of Bcl3 $^{-/-}$ mice compared with that in WT controls, whereas no differences were observed during the early phase after elicitation. In addition, a higher proportion of the infiltrating T cells in Bcl3 $^{-/-}$ mice stained positive for IFN- γ . The authors also demonstrate that various inflammatory chemokines, such as CXCL2, CXCL5, CXCL9, and CXCL10, were upregulated at the transcriptional level in allergen-treated ears at 48–96 h after challenge. These chemokines are all known to be inducible by IFN- γ (Fig. 1) [14]. Whereas CXCL2 and CXCL5 mainly attract neutrophils, CXCL9 and CXCL10 are predominantly responsible for the attraction of effector T cells expressing the chemokine receptor CXCR3 [15]. The findings of an inhibitory role of Bcl-3 in CHS described by Tassi et al. [4] are somewhat unexpected, as a previous study in humans indicated a

pathogenic role of Bcl-3 in the development of atopic dermatitis [16]. There, it was shown that Bcl-3 was upregulated in lesional skin regions of atopic dermatitis patients, and that silencing Bcl-3 enhanced the production of anti-microbial peptides (AMPs) in primary human keratinocytes stimulated with IL-4 and IL-13, suggesting that Bcl-3 negatively regulates AMP production [16]. The dominance of Th2 cytokines is characteristic of the acute phase of atopic dermatitis and reduced production of AMPs may explain the high susceptibility of these patients for superficial infection with *Staphylococcus aureus* [17]. The CHS model investigated by Tassi et al. [4], on the other hand, is characterized by enhanced production of the Th1/Tc1 cytokine IFN- γ , which may explain the different role of Bcl-3 in these two different forms of allergic skin reactions. As a proinflammatory chemokine involved in the pathogenesis of both CHS and atopic dermatitis we previously identified CCL17 that not only promotes the influx of effector T cells into the lesional skin, but also participates in the initiation of DC emigration from the skin to the draining lymph nodes after sensitization [18, 19].

Based on the observation that complete deficiency of Bcl-3 enhances skin inflammation in CHS, Tassi et al. [4] went on to study the cell-type specific functions of this transcriptional regulator. The authors first investigated the function of Bcl-3 in hematopoietic versus nonhematopoietic cells by generating bone marrow chimeras. These experiments clearly showed that Bcl-3 deficiency in radioresistant cells is responsible for the prolonged ear swelling response observed in Bcl-3-deficient mice. When Bcl-3-deficient bone marrow cells were transplanted into WT recipients, the CHS reaction was slightly ameliorated, in line with previous findings by the same group showing that mice harboring a DC-specific deletion of Bcl-3 develop reduced ear

swelling responses already at 24 h past challenge [20]. In this case, deletion of Bcl-3 was achieved by crossing Bcl-3^{flox} mice to the CD11cCre line, which besides DCs may also delete floxed target genes in a sizeable proportion of T cells [21]. Thus, the Bcl-3-dependent downregulation of the CHS response in radioresistant cells appears to dominate over a possible enhancement of the hypersensitivity response via the action of Bcl-3 within bone marrow-derived myeloid cells. To identify the radioresistant cell type responsible for this effect, Tassi et al. [4] analyzed two further conditional Bcl-3 knockouts: keratinocyte-specific Bcl-3 deletion, using K5-Cre mice, or Langerhans cells (LCs)-specific Bcl-3 deletion, using Langerin-Cre mice. Interestingly, the absence of Bcl-3 in keratinocytes was found to be responsible for the enhanced production of the IFN- γ -dependent chemokines CXCL9 and CXCL10 in the skin (Fig. 1). The identification of keratinocytes as the main producers of CXCL9 and CXCL10 was further supported by quantification of *cxcl9* and *cxcl10* in epidermal and dermal cell isolates from oxazolone-treated WT and Bcl-3^{-/-} mice. Also in this case, transcription of *cxcl9* and *cxcl10* was mainly observed in the epidermis. In line with the enhanced production of these T-cell-attractant chemokines, the ear skin of keratinocyte-specific Bcl-3 knockout mice was more heavily infiltrated by IFN- γ -producing CD8⁺ T cells than that of WT controls. In contrast, neutrophil inflammation was similar in both cases. When analyzing the LC-specific knockouts, the authors did not observe changes in the responsiveness to oxazolone-induced CHS at all. Thus, the phenotype of global Bcl3-deficient mice was partially recapitulated by the keratinocyte-specific knockout but not by the LC-specific knockout. The enhanced ear swelling response of complete Bcl-3^{-/-} mice, however, was not observed in the conditional knockouts, indicating that there must be other radioresistant cell types in the skin in which Bcl-3 is of functional importance for CHS development. Tassi et al. speculate that such cells may be dermal fibroblasts, adipocytes, endothelial cells, or perhaps a radioresistant subset of dermal DCs. Another possibility would be that dendritic epidermal T cells, which are in close physical contact with keratinocytes and which have been shown to play a key role in CHS [22], are also involved. In fact, dendritic epidermal T cells were previously demonstrated to be radioresistant [23]. An alternative explanation may be that several different radioresistant cells types act together to downregulate the CHS response in a Bcl-3 dependent manner (Fig. 1).

Given that they are positioned at the outermost layer of the skin, keratinocytes represent important sensors of skin irritants, UV light, injury, and infection [24]. They are known to express a large variety of pattern recognition receptors that recognize pathogen-associated molecular patterns as well as exogenous and endogenous danger signals. Contact sensitizers such as oxazolone and dinitrofluorobenzene trigger the production of reactive oxygen species in keratinocytes that, in turn, lead to degradation of hyaluronic acid and stimulation of TLRs, as well as release of ATP and activation of the inflammasome [2, 3]. As a result, keratinocytes exposed to such external stresses are capable of activating innate immune mechanisms and driving the production of proinflammatory cytokines and chemokines. Besides CXCL9

and CXCL10, CCL8 has also been described recently as an IFN- γ -inducible chemokine in keratinocytes that is upregulated in the context of CHS. This was demonstrated by analysis of keratinocyte-specific conditional knockout mice for the cannabinoid receptor-1 that is otherwise mainly expressed in neurons to control pain perception and facilitate the extinction of aversive memories [25]. Exposure of keratinocytes to UV light has been shown to activate innate immune mechanisms through the release of high mobility group box 1, a ligand of TLR4, which acts on myeloid cells in a MyD88-dependent manner and mainly enhances the influx of neutrophils into the skin [26]. Furthermore, UV light has been shown to enable the production of vitamin D3 from 7-dehydrocholesterol in the skin, which in turn enhances the release of AMPs, a process that is also regulated by Bcl-3 [16]. Thus, keratinocyte activation by environmental danger signals critically contributes to immune defenses in the skin, but needs to be properly controlled to prevent adverse immune reactions.

In conclusion, from the work of Tassi et al. [4] Bcl-3 can be added to the list of important modulators of allergic immune reactions in the skin, with specific functionality in radioresistant cells, including keratinocytes. The actual molecular partners of Bcl-3 in different cell types remain to be elucidated, as well as the manner in which these factors influence the production of innate immune mediators at the transcriptional and posttranscriptional level.

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Abbreviations: AMP: anti-microbial peptide · Bcl-3: B-cell lymphoma-3 · CHS: contact hypersensitivity · LC: Langerhans cell

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