

In summary, although a complete understanding of the mechanisms leading to palmoplantar keratoderma and nail dystrophy in PC has yet to be achieved, *Krt16*-null mice provide additional insights into this process and highlight the importance of *KRT16* in the normal structure and function of palmoplantar epithelia.

CONFLICT OF INTEREST

The author states no conflict of interest.

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See related article on pg 1392

IL-33: A Novel Danger Signal System in Atopic Dermatitis

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IL-33 is a newly recognized cytokine of the IL-1 cytokine family that has recently been attributed to the epithelial “alarmin” defense system. IL-33 is released by the epithelial cells in various tissues and organs, including keratinocytes, endothelial cells, and immune cells. Recent reports have suggested that IL-33 might be a critical part of the innate immunity, although its precise role is as yet poorly understood. In several organs, IL-33 appears to drive T helper type 2 (Th2) responses, suggesting roles in allergic and atopic diseases, as well as in fibrosis. IL-33 exerts its effects by activating the ST2 (suppression of tumorigenicity 2)/IL-1 α R receptor on different types of cells, including mast cells and Th2 cells. The ST2 receptor is either expressed on the cell surface or shed from these cells (soluble ST2, sST2), thereby functioning as a “decoy” receptor. After binding to its receptor, IL-33 activates NF- κ B, suggesting that it regulates the outcome of diseases such as atopic dermatitis. On the other hand, several studies have reported on the inhibitory effects of sST2 in inflammatory and fibrotic diseases, suggesting that IL-33/ST2 is a unique cytokine with potential pro- and anti-inflammatory effects.

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Atopic dermatitis (AD) is a common chronic inflammatory skin disease characterized by an early T helper type 2

(Th2) “immune signature”: patients suffer from relapsing eczematous and occasionally generalized (erythroderma) lesions

associated with severe pruritus (Bonness and Bieber, 2007; Boguniewicz and Leung, 2011). Scratching reactions to pruritus typically exacerbate the inflammatory skin reactions (Hong *et al.*, 2011). The key events in AD may be subdivided as an interplay among (1) infiltrating immune cells (Th2 cells and—later—Th1 cells, macrophages, dendritic cells, mast cells, and eosinophils); (2) skin-resident keratinocytes and endothelial cells; and (3) activated (“hypersensitive”) peripheral sensory nerves. The multicellular action is believed to orchestrate disease onset and progression (Steinhoff *et al.*, 2006; Cevikbas *et al.*, 2007). Unfortunately, current AD treatments, which suppress inflammation broadly (e.g., steroids, cyclosporin A), are hampered by effects on other cells and pathways that are unrelated to the disease.

The adaptive and innate immune systems have important and bidirectional roles in the pathophysiology of AD (Bieber, 2008; Elias and Steinhoff, 2008). Cytokines such as IL-4 and IL-13 regulate proinflammatory responses of the adaptive immune response in early phases of AD by regulating Th2 activation; thus, they are considered optimal targets for therapies. Keratinocytes, however, as part of the innate immune defense, also contribute to the inflammatory reactions and immune responses in AD by regulating the release of cytokines, chemokines, proteases, and bioactive lipids. Upon stimulation by allergens, toxins, or infectious agents, keratinocytes are capable of initiating a cross-talk between the adaptive and innate immune responses by activating T cells in patients with AD through the release of key molecules (Homey *et al.*, 2006). Thus, cytokines such as IL-25 and chemokines such as TSLP (thymic stromal lymphopoietin) or CCL27 have important roles in this interactive network (Carmi-Levy *et al.*, 2011).

Recent evidence points to a role for the IL-33/ST2 (suppression of tumorigenicity 2) pathway in epithelial integrity, allergic immune responses, inflammation, autoimmunity, and fibrosis, which are just several examples (Moussion *et al.*, 2008; Ivanov *et al.*, 2010; Rankin *et al.*, 2010). In skin, the functional role of this newly recognized IL-33/ST2 pathway has gained attention. The

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findings thus far indicate that the release of IL-33 by keratinocytes, endothelial cells, or immune cells activates the IL-33 receptor ST2 on keratinocytes, fibroblasts, mast cells, or other immune cells, leading to the expression of factors implicated in several inflammatory pathways (Pushparaj *et al.*, 2009; Liew *et al.*, 2010). This IL-33/ST2-induced immune regulation may have a crucial role in adaptive as well as innate immune responses in skin.

IL-33 has important roles in the pathogenesis of several Th2-biased

inflammatory conditions and allergic reactions (Verri *et al.*, 2008; Figure 1). An alternative transcript from the ST2 locus encodes a soluble form of the ST2 receptor (sST2), which acts as a natural IL-33 antagonist (decoy receptor). IL-33-dependent activation of ST2 signaling leads to activation of the signaling pathways signal transducer and activator of transcription 5, mitogen-activated protein kinase, Akt, and NF- κ B (Guo *et al.*, 2009; Ivanov *et al.*, 2010; Ali *et al.*, 2011; Funakoshi-Tago *et al.*, 2011), most of which ultimately have

roles in the pathogenesis of AD. The mature form of IL-33 is released into the cytoplasm and subsequently stimulates T cells, mast cells, or keratinocytes. Similar to IL-1, full-length IL-33 can act as a transcription factor by trafficking into the nucleus, where it modulates several inflammatory responses (Carriere *et al.*, 2007).

Intriguingly, IL-33 expression is upregulated in keratinocytes and endothelial cells in AD (Oboki *et al.*, 2010). Injection of recombinant IL-33 into mouse skin *in vivo* is sufficient to cause infiltration of T cells, macrophages, and eosinophils, all of which are immune cells that express the functional ST2/IL-1R4 receptor complex (Kroeger *et al.*, 2009; Rankin *et al.*, 2010; Anthony *et al.*, 2011; Eiwegger and Akdis, 2011; Ohno *et al.*, 2011; Zaiss *et al.*, 2011). Furthermore, IL-33 stimulation leads to the release of Th2-associated mediators, suggesting that IL-33 might fulfill a crucial role in Th2-associated diseases such as AD. In summary, considerable evidence points to a key contribution of the IL-33/ST2 pathway in inflammatory skin diseases, including AD. In the future, it will be important to understand the precise roles of the different subforms of IL-33 (secreted, intracellular) and ST2 (transmembrane, decoy) in skin.

Savinko *et al.* (this issue, 2012) investigated the expression profiles of IL-33 and ST2 in different mouse models of atopic-like dermatitis (AD), emphasizing a regulatory role for this novel cytokine pathway. In a translational setting, the authors also quantified the messenger RNA levels for IL-33 and ST2 in lesional and nonlesional human skin. Via immunohistochemistry, the authors restricted the distribution of IL-33⁺ cells to suprabasal keratinocytes. The ST2⁺ cell population was found to be dermal and epidermal in origin, although a precise characterization of the ST2⁺ dermal cells is still lacking. Different allergens, when applied topically (ovalbumin, house dust mites, or staphylococcal enterotoxin B) to mice, led to the upregulation of IL-33 and ST2 messenger RNA expression. These results indicate that IL-33 as well as its receptor may be induced in AD when exposed to AD trigger factors. Whether these results reflect the human situation remains unknown. Intriguingly, topical

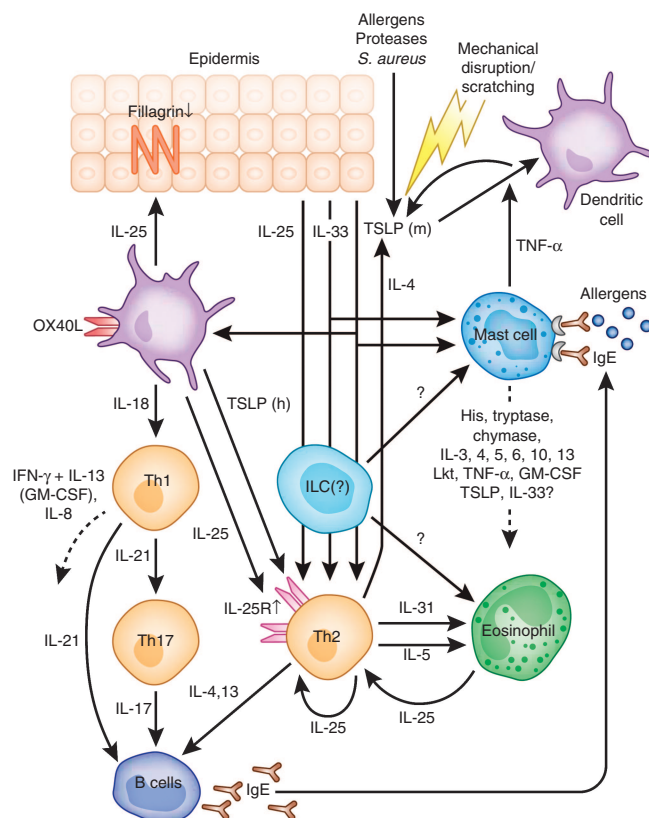


Figure 1. Potential role of IL-33 and ST2 (suppression of tumorigenicity 2) in skin inflammation and pathophysiology of atopic dermatitis. Trigger factors such as allergens, bacteria, mechanical injury, and exogenous proteases induce the release of IL-33, IL-25, and thymic stromal lymphopoietin (TSLP) from keratinocytes. IL-33, alone or in combination with other cytokines and chemokines, activates mast cells, dendritic cells, and (potentially) lymphocytes of innate lineage in inflamed skin. Activation of mast cells regulates the function of dendritic cells, eosinophils, T cells, and nerves (not shown) via release of cytokines, chemokines, growth factors, proteases, and leukotrienes, for example. Via the IL-33-induced release of IL-25 and IL-18, dendritic cells regulate the function of T helper type 2 (Th2) cells and B cells (early) and (probably later) Th1 cells. Via IL-33 induction, innate lymphoid cells (ILCs) regulate Th2-driven immune responses in various organs. The role of these potentially important cells in Th2 regulation has not been verified in skin. ILCs have been described to be important in the early stage of immune signaling and probably enhance the Th2 immune response pathway in the skin. The arrows illustrate the interaction among the various immune cells and their cell-specific secretion products involved in inflammatory skin diseases, including atopic dermatitis. Thus, IL-33 may be involved in orchestrating early inflammatory responses in the onset and perpetuation of atopic dermatitis. His, histamine; Lkt, leukotriene; OX40 (gp34), a member of the TNF superfamily; TNF- α , tumor necrosis factor- α (Modified from Carmi-Levi *et al.*, 2011).

Clinical Implications

- The newly recognized cytokine IL-33 influences atopic dermatitis variously, up and down.
- The mechanism of action establishes IL-33/ST2 as a potential target to treat atopic dermatitis.
- Lymphocytes of innate immunity may be regulated by IL-33 in human skin disease.

treatment with calcineurin inhibitors such as tacrolimus, a topical drug used widely to treat AD, reduced the expression levels of both IL-33 and ST2. Combined stimulation with pro-inflammatory cytokines such as tumor necrosis factor (TNF)- α and IFN- γ caused an increase in IL-33 protein expression in cultured fibroblasts and HaCaT keratinocytes. The possibility of translating these observations to humans is unlikely because IL-33 appears to be an early cytokine in the inflammatory cascade, and neither TNF- α nor IFN- γ has important roles in the early phases of AD. Thus, the role of the IL-33/ST2 pathway in early and late inflammatory responses remains uncertain, and its impact during the different phases of inflammation and immune defense remains controversial, depending on the tissues and species being studied.

Savinko *et al.* (2012) also emphasized a potential interaction between keratinocyte-derived IL-33 and mast cells, which are critical in AD pathogenesis, showing that bone marrow-derived mast cells activated by IgE⁺ allergens increase the expression levels of ST2 and IL-33. The report analyzes the expression profiles of IL-33 and ST2 for a well-chosen set of allergens believed to be important trigger factors for AD.

Thus far, IL-33 has been implicated in pro- as well as anti-inflammatory processes in various tissues and cells (Haraldsen *et al.*, 2009; Kroeger *et al.*, 2009). Hence, a key issue is to identify when IL-33 acts as a pro-inflammatory molecule and when it acts as an anti-inflammatory molecule during different stages of an immune response. For example, in the intestine, IL-33 stimulates the induction of Th2-associated cytokines (IL-4, IL-9, IL-13) during parasite infection and prevents Th1-polarized cell responses. Interestingly, exogenous IL-33 also induces TSLP messenger RNA,

another early signaling molecule in the epidermis (Humphreys *et al.*, 2008). In human mastocytoma cells and bone marrow-derived mast cells, IL-33 induces IL-1 β , TNF- α , MCP-1 (monocyte chemoattractant protein-1), and PGD₂ (prostaglandin D₂) production and increases IL-6 and IL-1 β messenger RNA expression *in vitro* (Moulin *et al.*, 2007). In some of these models, the proinflammatory effects of IL-33 are inhibited by sST2 acting as a decoy receptor.

Although poorly studied, new evidence points to an important role for the IL-33/ST2 pathway in many skin diseases, including AD in humans (Pushparaj *et al.*, 2009). In a mouse model of skin inflammation and UVB-induced dermatitis, IL-33 levels were found to be enhanced at both the RNA and protein levels. *In vitro*, UVB and oxPAF (oxidized alkyl acyl phosphocholines) increased IL-33 expression in keratinocytes and fibroblasts. Notably, IL-33 application in a model of Th1-mediated acute contact hypersensitivity markedly suppressed its proinflammatory effects. Thus, IL-33 may be an important early danger signal in response to UV radiation (Byrne *et al.*, 2011).

An exciting recent observation was the characterization of innate lymphoid cells (ILCs) as an important link in the communication between innate and adaptive immunity (Saglan, 2011). ILCs are crucial in organizing inflammation and immunity in the lung, intestine, and adipose tissue. Together, these *in vitro* and *in vivo* findings implicate a role for IL-33 as an early danger-sensing molecule during the impairment of tissue integrity, as observed in inflammation, allergy, and tissue injury (Figure 1). For example, Monticelli *et al.* (2011) showed that ILCs in the lung expressing CD90, IL-2, CD25, CD127, and ST2 accumulate after influenza A infection. Notably,

depletion of ILCs resulted in loss of epithelial integrity and impaired remodeling and airway function, at least in part via ST2 activation. This indicates that ILCs may be critical for restoring epithelial integrity and tissue homeostasis, partly via ST2 activation by IL-33.

The work by Savinko *et al.* (2012) expands our understanding of the complex interaction between the skin's innate and adaptive immune responses. Nevertheless, important questions in our understanding of the IL-33/ST2 signaling system in skin inflammation and immune regulation remain. (1) Where are IL-33 and ST2 expressed under physiological and pathophysiological conditions in the skin? (2) How does this cytokine system signal during acute and chronic inflammation? (3) When does IL-33 exert its pro- and anti-inflammatory effects, and when and why does the switch occur? (4) How is IL-33 regulated in skin cells? (5) When is IL-33 present in its mature form in skin cells? (6) What is the physiological role and what is the pharmacological benefit of sST2 release? (7) What is the functional role of IL-33 and ST2 in the early and late phases of AD? Answers to these questions will open a new chapter in our understanding of the complex interplay of the innate and adaptive immune defense in AD, opening possibilities for novel targeted therapies.

CONFLICT OF INTEREST

The author states no conflict of interest.

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See related article on pg 1363

Toward Personalized Medicine in Scleroderma: Classification of Scleroderma Patients into Stable “Inflammatory” and “Fibrotic” Subgroups

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There is no universally agreed-upon treatment for the fibrosis of scleroderma. Recently, much information has been generated relating to the fundamental mechanisms underlying this disease. Partly based on these observations, both anti-inflammatory and anti-fibrotic agents have been considered as possible therapies. However, this information has not been successfully translated into clinical practice. In this issue, Pendergrass *et al.* use genome-wide expression profiling to provide valuable insights into scleroderma. Previously, the authors showed that morphea and “limited” scleroderma patients and a small subset of diffuse scleroderma (dSSc) patients express an “inflammatory” profile, whereas the majority of dSSc patients express a “fibroproliferative” profile. In the current study, the investigators show that the gene expression profile of these patients is fixed over time; i.e., in contrast to a previously held belief, the inflammatory patients do not go on to become fibrotic, and vice versa. These data suggest that expression profiling might be used to design clinical trials for scleroderma. The inflammatory patients might be treated with anti-inflammatory agents, whereas fibroproliferative patients might be treated with antifibrotic agents.

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A connective-tissue disorder of unknown etiology, scleroderma (also called systemic sclerosis or SSc), is a multisystem

disease characterized by the presence of autoantibodies, vascular damage, and organ fibrosis (Abraham and Varga,

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