comparison with normal skin. The role of Th2 cytokines was then confirmed using keratinocyte primary cultures treated with IL-4/IL-13. In contrast, gamma-INF treatment did not modulate alpha toxin–mediated cytotoxicity. IL-4/IL-13 augmentation of alpha toxin–mediated cytotoxicity was shown to be due to the ability of these Th2 cytokines to decrease the levels of acid sphingomyelinase, the enzyme that cleaves sphingomyelin into ceramide and phosphocholine. The ability of exogenous sphingomyelinase or phosphocholine to reverse the alpha toxin toxicity not only confirmed the mechanisms but also provided the rationale for possible therapeutics.

The authors also demonstrated in a recent companion report that the epidermal barrier protein filaggrin also has an important role in the regulation of acid sphingomyelinase, and that deficiency in filaggrin predisposes for increased alpha toxin–mediated cytotoxicity (Brauweiler *et al.*, 2013). Relevant to Th2 cytokines, IL-4 and IL-13 have also been shown to inhibit filaggrin expression (Howell *et al.*, 2009). Thus, one can envision a positive feedback pathway by which Th2 cytokines not only provide a permissive environment for *S. aureus* but also enhance the effects of staphylococcal products. These findings are compatible with the notion that strategies that remove bacteria, such as dilute bleach baths, can improve AD skin disease (Huang *et al.*, 2009).

In summary, the interplay between Th2 cytokines and *S. aureus* has high significance for understanding AD. Indeed, the majority of AD flares involve *S. aureus* in some capacity. New insights into the exact mechanisms by which staphylococcal exposure can worsen AD could allow improved and novel treatments for this common skin disease and its significant morbidities.

CONFLICT OF INTEREST

The author states no conflict of interest.

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A Possible Role for IL-17A in Establishing Th2 Inflammation in Murine Models of Atopic Dermatitis

Nikhil Dhingra[1,2] and Emma Guttman-Yassky[1,3]

Atopic dermatitis (AD) is associated with the effects of T helper type 2 (Th2) and Th22 cytokines. Recent studies, however, have also implicated Th17 in acute AD. Functional studies of Th2 and Th22 cytokines revealed their roles in generating molecular changes during AD; IL-17A's role, however, has yet to be defined. The report by Nakajima *et al.* (this issue) begins to define that role by demonstrating IL-17A's ability to induce Th2 inflammation in acute disease.

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Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by acute and chronic stages. The pathogenesis of AD is still unclear; both immune- and barrier-driven mechanisms have been demonstrated in murine models and in human disease. AD has been recognized predominantly as a T helper type 2 (Th2)/Th22-centered disease (Guttman-Yassky *et al.*, 2011). Lesional AD skin is characterized primarily by increased expression of Th2 cytokines and chemokines (IL-4/IL-13, chemokine (C-C motif) ligand (CCL)17, OX40), Th22-related markers (IL-22), as well as Th1- (chemokine (CXC motif) ligand (CXCL10)), Th17- (Elafin/PI3, CXCL1), and Th17/Th22-mediated S100

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Clinical Implications

- Nakajima *et al.* (2014) demonstrated IL-17A-mediated induction of Th2 inflammation in the setting of hapten exposure or barrier disruption in murine models, thereby introducing a new facet into AD pathogenesis models.
- Their finding provides a better understanding of the primary immunological processes that initiate acute and chronic disease.
- Ongoing studies in humans using IL-4- and IL-17-targeted biologics will provide additional insights into the translatability of this finding.

epidermal responses. Although the pathogenic role of Th17 in psoriasis is well established and supported by disease antagonism with anti-IL-17 biologics (i.e., brodalumab, ixekizumab, and secukinumab), its role in the pathogenesis of AD remains unclear. Although IL-17 is increased in skin and peripheral blood in patients with acute disease (Gittler *et al.,* 2012), IL-17 and its associated antimicrobial peptides are much lower than in psoriasis, possibly explaining the increased risk of infections in AD (reviewed in Guttman-Yassky *et al.,* 2011). A recent study by our group compared nonlesional tissue with that of acute and chronic lesions from the same patient, which revealed increased expression of IL-17 and several associated markers (CCL20, PI3) in acute AD compared with non-lesional skin. Interestingly, levels remained insignificantly changed as disease progressed to the chronic stage (Gittler *et al.,* 2012; Figure 1). Obtaining a functional understanding for IL-17A in AD has remained elusive until now. In this issue, Nakajima *et al.,* (2014) used two murine models of AD, including hapten-mediated induction and flaky-tailed mice with spontaneous lesions, in the setting of IL-17A deficiency to evaluate its role in disease pathogenesis.

Th2 induction by IL-17A in a murine model of AD-like inflammation

Using both models, Nakajima *et al.* (2014) demonstrated that IL-17A deficiency attenuated Th2 induction during the acute phase of inflammation, implicating IL-17A as an inducer or modifier that drives IL-17A T cells toward Th2 in the early inflammation of susceptible skin. The hapten-induced mouse model of AD relies on the concept that chronic hapten exposure across an impaired barrier establishes a pro-inflammatory milieu (the "hapten-atopy hypothesis"; McFadden *et al.,* 2011) dominated by Th2 T cells, mimicking the inflammation in AD skin. The hapten model by Nakajima *et al.* (2014) may explain how an immune response to an external allergic agent, via IL-17A-mediated Th2 induction, ultimately polarizes toward the acute AD cytokine milieu that has been described previously. In addition, the demonstration by Nakajima *et al.* (2014) of similar immune findings in barrier-deficient mice with spontaneous AD-like lesions helps to bridge our understanding of why some patients develop lesions only in the setting of a sensitizer, whereas others develop them spontaneously. Their finding that contact allergen-triggered disease and barrier deficiency both result in similar IL-17A-mediated induction of Th2 inflammation may explain how this clinical phenomenon occurs.

Interestingly, in a recent report, Newell *et al.* (2013) described the use of the potent hapten dinitrochlorobenzene to study contact sensitization in humans, revealing an interesting dichotomy between patients with AD and volunteers without AD. Those with a history of AD exhibited preferential Th2 induction, whereas those without AD exhibited a Th1 response primarily. Thus, it can be postulated that in human AD, an allergen penetrating across a defective barrier may trigger low-grade IL-17A release; with ongoing stimulation by chronic happen exposure, it may induce Th2-polarizing molecules (thymic stromal lymphopoietin, CCL17), promoting naïve T cells toward Th2 and establishing the predominant immune polarization of AD. Interestingly, a similar concept may be applied to the role of *Staphyloccoccus aureus*, another implicated extrinsic agent, in triggering AD exacerbations. *S. aureus* superantigens, like the haptens in the study by Nakajima *et al.* (2014), were also demonstrated to induce IL-17A expression (Boguniewicz and Leung, 2010), potentially suggesting a common molecular link between the two major external inducers of disease flares.

Limitations of the murine contact hypersensitivity model and our current understanding of IL-17 signaling introduce new questions

The AD mouse models used by Nakajima *et al.* (2014) have several inherent limitations with respect to their translatability to humans. Specifically, at present, no single mouse model represents acute and chronic AD with true accuracy; rather, these models are able to represent select characteristics of the disease process and generate AD-like clinical and molecular inflammation. The murine contact hypersensitivity model, for instance, can only be induced via potent sensitizing agents such as dinitrochlorobenzene, which have an inherent irritating capacity and thus induce nonspecific immune activation (i.e., of the innate immune response) in addition to antigen-specific activation. Conversely, clinically relevant sensitizers that cause allergic contact dermatitis, and may trigger human AD, do not exhibit an initial irritant response, thus making it difficult to translate the generated immunological data fully to human models. Another important limitation relates to γδ T cells, which Nakajima *et al.* (2014) found to be the chief producers of IL-17A in their study; the role of these T cells in human IL-17 production is unknown at present, and thus it is unclear whether a similar mechanism may exist in humans.

Only a few studies prior to those by Nakajima *et al.* (2014) evaluated the human effector interplay between IL-17A and the Th2 pathway, particularly in skin disease, and a select subset from other tissue types reported results contradictory to those in this study. One such work, by Xu *et al.* (2010), on cultured nasal epithelial cells revealed an inhibitory effect of IL-17A on thymic stromal lymphopoietin expression, whereas Th2-sourced IL-25 (also known as IL-17E) had the opposite effect. Studies in skin too have produced dissimilar results from those by Nakajima

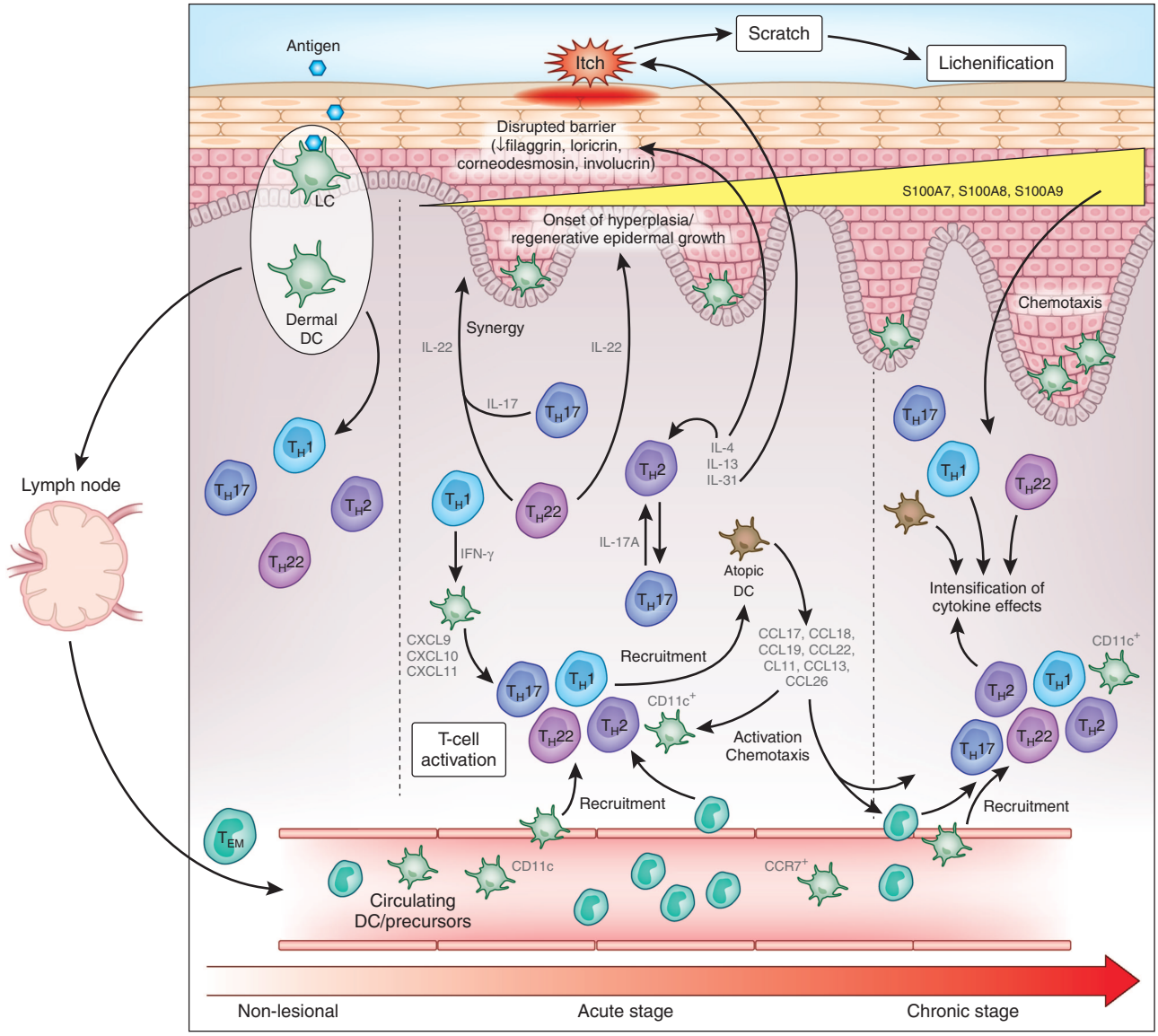

Figure 1. A schematic illustration of the initiation of acute atopic dermatitis (AD) and progression to chronic skin lesions. Non-lesional AD skin lesions show some immune infiltrates that produce inflammatory mediators, which may contribute to a defective epidermal barrier. Barrier defects lead to penetration by epicutaneous antigens that encounter Langerhans cells in the epidermis and dermal dendritic cells (DCs) in the dermis, inducing marked immune activation and recruitment of inflammatory cells in acute AD lesions. The finding by Nakajima *et al.* (2014) regarding the role of IL-17A may potentially bridge the gap between non-lesional skin following antigen penetration and onset of acute disease, where T helper type 17 (Th17) T cells have been identified in limited quantities. This relatively small Th17 activity could potentially lead to the marked activation of Th2 axis in acute disease onset; Th22 activity also increases. A progressive activation of the Th2, Th22, and Th1 pathways is characteristic of the chronic stage of AD; Th17 activity remains constant from acute disease. Cytokines (i.e., IL-4 and IL-13) and chemokines (i.e., chemokine (C-C motif) ligand (CCL)17, CCL18, CCL19, chemokine (CXC motif) ligand (CXCL)9, CXCL10, and CXCL11) produced by various T cells and DCs induce further activation and recruitment of additional immune cells. With the onset of acute disease, Th22 cells release IL-22, which induces epidermal hyperplasia and, synergistically with the Th17 cytokine IL-17, drives an abrupt increase in a subset of terminal differentiation genes, specifically the S100A7, S100A8, and S100A9 proteins. The increases in these barrier proteins contrast with the uniformly disrupted epidermal differentiation gene products (e.g., filaggrin, loricrin, and corneodesmosin) throughout non-lesional, acute, and chronic AD skin. The Th2 and Th22 cytokines contribute to inhibition of the terminal differentiation proteins. IL-31 is abruptly upregulated in acute disease, potentially reflecting its role as an itch mediator in patients with AD. Figure and legend adapted and modified from Gittler *et al.* (2012) with permission from Elsevier/RightsLink.

et al. (2014). Bogiatzi *et al.* (2012) demonstrated, using human skin explants and cultured dendritic cells, that IL-17A induced marked decreases in thymic stromal lymphopoietin and Th2-related cytokine production. Eyerich *et al.* (2009) complicated this picture further, postulating that IL-4 was capable of inhibiting the IL-17 pathway as well.

A final point of consideration is revealed in recent studies that identified a subset of Th2 T cells capable of producing IL-17A itself. Wang *et al.* (2010) demonstrated the presence of these IL-4- and IL-17-producing Th2 T cells in peripheral blood of allergic asthmatics. It can be postulated that IL-17A produced by these dual-function cells may signal back on

naive T cells, augmenting Th2 activity. Interestingly, they reported that these cells could be stimulated to produce IL-17 by IL-1β, IL-6, and IL-21 but not by IL-23, suggesting a distinct mechanism from classical Th17-mediated release. The same study found that in murine models of allergic asthma, mice with allergen-specific IL-17-producing Th2 cells exhibited increased airway inflammation following allergen exposure, suggesting some correlation between clinical disease activity and stimulation of these IL-17-producing Th2 T cells. Similarly, in skin, Eyerich *et al.* (2009) used eczema samples and identified that approximately one-third of IL-17-producing cells exhibited a concurrent Th0 or Th2 profile (both IL-4-producing without or with IFN-γ, respectively). Thus, additional studies, based on the work by Nakajima *et al.* (2014), will be required to evaluate the possibility that IL-17A is itself being sourced from a subset of Th2 T cells rather than from the classically presumed Th17 cells.

Upcoming targeted therapeutics for AD may reveal further insights into the role of cytokine interplay in disease pathogenesis

AD research has recently entered a new era in which our collective understanding of the immune mechanisms driving disease has led to the development and testing of agents targeting key molecules in pathogenic pathways. A clinical trial that is currently being conducted by our group testing ustekinumab (anti-IL-23p40) (clinicaltrials.gov, NCT01806662) in AD patients may help elucidate the effects of IL-17A inhibition on Th2 cytokine induction. By inhibiting Th17 T-cell activation (and thus significant IL-17 production) with this biologic, we will be able to evaluate the posttreatment effects of such a blockade on all the major inflammatory axes, including Th2 T cells and their associated markers. In doing so, our study may bridge the murine work by Nakajima *et al.* (2014) to humans, potentially providing insights into whether IL-17 inhibition truly reduces Th2 activity. Conversely, dupilumab (clinicaltrials.gov, NCT01859988), a mAb that inhibits IL-4Rα, dually blocking IL-4 and IL-13 signaling may shed light on the effects of Th2 inhibition on the IL-17 pathway genes in addition to the other major T-cell axes. Moving forward, a bedside-to-bench approach with pathway-targeted therapeutics will enable us to not only test the efficacies of these drugs but also potentially unveil additional novel targets in the major inflammatory pathways. The work by Nakajima *et al.* (2014) in this issue of the journal, however, presents a fascinating model for the initiation of Th2 inflammation in AD, providing a potential therapeutic target and an exciting clue into the early stages of the slowly unraveling AD pathogenesis model.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Oxidative Stress and Vitiligo: The Nrf2–ARE Signaling Connection

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Hypersensitivity of epidermal melanocytes to oxidative stress is known to contribute to vitiligo pathogenesis. Molecular mechanisms that connect melanocyte redox homeostasis to the complex disease phenotype are not fully understood. Jian *et al.* show that vitiligo melanocytes have impaired nuclear factor erythroid 2–related factor 2 (Nrf2)–antioxidant response element signaling and decreased activation of the antioxidant enzyme system. In patients with vitiligo, higher serum levels of IL-2 correlate with lower levels of hemeoxygenase-1, a product of the Nrf2 target gene.

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Degeneration of melanocytes, the cellular source of melanin pigment, is the pathologic hallmark of depigmented macules in patients with vitiligo. Vitiligo is now recognized as a disorder of multifactorial etiology culminating in

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