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Mechanisms of Contact Sensitization Offer Insights into the Role of Barrier Defects vs. Intrinsic Immune Abnormalities as Drivers of Atopic Dermatitis

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Atopic dermatitis (AD) is a common inflammatory skin disease characterized by wet, oozing, erythematous, pruritic lesions in the acute stage and xerotic, lichenified plaques in the chronic stage. It frequently coexists with asthma and allergic rhinitis, sharing some mechanistic features with these diseases as part of the “atopic march.” Controversy exists as to whether immune abnormalities, epidermal barrier defects, or both are the primary factors responsible for disease pathogenesis. In AD patients, there is often a coexisting irritant contact dermatitis (ICD) or allergic contact dermatitis (ACD) that is sometimes clinically difficult to distinguish from AD. ACD shares molecular mechanisms with AD, including increased cellular infiltrates and cytokine activation (Gittler *et al.*, 2013). In this issue, Newell *et al.* (2013) used an experimental contact sensitization model with dinitrochlorobenzene (DNCB) to gain insight into the unique immune phenotype of AD patients.

Journal of Investigative Dermatology (2013) 133, 2311–2314. doi:10.1038/jid.2013.239

Epidermal barrier defects characterize lesional and nonlesional AD skin

The stratum corneum, including terminal differentiation proteins such as filaggrin (FLG), corneodesmosin, and loricrin, is a first-line defense against irritants and allergens. The genomic expression of key barrier molecules, which comprise the epidermal differentiation complex on chromosome 1q21, have been previously shown to be downregulated in AD patients in both lesional and nonlesional skin (Suárez-Fariñas *et al.*, 2011).

Furthermore, frequent mutations in the *FLG* gene (found in up to 30–50% of AD patients) have been associated with the severity of AD (as identified by the Scoring of AD (SCORAD) index). These differentiation abnormalities contribute to the barrier defect in AD, ultimately resulting in increased transepidermal water loss, xerosis, and greater penetration of various agents (Gittler *et al.*, 2013). In humans, *FLG* deficiency has been linked to increased risk of the other atopic diseases, as well as to greater

susceptibility to common triggers of AD (including allergens and microbes). Murine models with reduced *FLG* exhibit greater passive transfer of protein allergens and reduced thresholds to irritants (Irvine *et al.*, 2011). These studies provide the basis for the “outside-in” hypothesis of AD, which states that transfer of external triggers across a dysfunctional barrier elicits the disease’s characteristic immune responses. Although *FLG* and other defects in the barrier have been linked to AD pathogenesis, there are notable limitations to this hypothesis. For example, an inverse correlation has been established between the expression levels of several terminal differentiation molecules and AD disease severity (as measured by the SCORAD index) (Suárez-Fariñas *et al.*, 2011). This raises the possibility of a reactive epidermal barrier to a primary immune insult. Furthermore, impressive reductions in key terminal differentiation molecules, extending far beyond *FLG*, have been found even in nonlesional AD skin. In addition, a majority of AD patients do not harbor *FLG* mutations, and even those with them have been shown to outgrow the disease (Guttman-Yassky *et al.*, 2011). Collectively, these observations suggest that barrier dysfunction is not the sole contributor to disease pathogenesis.

AD is primarily Th2 and Th22 polarized

The historical immune paradigm for AD characterized it largely as a T helper type 2 (Th2)-mediated disease with high levels of IL-4, IL-13, and Th2-polarizing chemokines (i.e., CCL17, CCL18, and CCL22). Recent work has implicated additional key Th2-associated cytokines and factors, including IL-31, thymic stromal lymphopoietin (TSLP), and OX40. Th2 signaling has been demonstrated to produce many of the molecular findings seen in AD skin, with the exception of the characteristic epidermal hyperplasia. Allergen-specific Th2 T cells can be found in AD but not in nonatopic controls (Arden-Jones *et al.*, 2007). More recently, Th22 T cells and their cytokine, IL-22, have been shown to have a key role in the pathogenesis of AD, potentially accounting for the increased epidermal thickness. Langerhans

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Clinical Implications

- Atopic dermatitis (AD) is T helper type 2 (Th2)-polarized and often co-occurs with contact dermatitis.
- Using contact sensitization, Newell *et al.* provide insights into the Th2 skewing of AD.
- Th2 skewing is independent of filaggrin status.

cells (LCs) and/or CD11c+ dermal dendritic cells (dDCs), which are up-regulated in AD, have been associated with both Th2 and Th22 polarization, possibly explaining the predominance of these T-cell subsets in the disease (Fujita *et al.*, 2011; Gittler *et al.*, 2013). AD has also been shown to have a Th1 component in the chronic phase, and a Th17 component in the acute stage (Guttman-Yassky *et al.*, 2011; Gittler *et al.*, 2013). Thus, concepts of the AD immune phenotype have become increasingly complex, with evidence for activation of several pathways beyond the Th2 axis (Figure 1a). Recently, the “background” or nonlesional disease phenotype has also been shown to display increased cellular infiltrates (i.e., T cells, DCs, and LCs), as well as increased expression of inflammatory mediators, compared with normal skin (Suárez-Fariñas *et al.*, 2011), possibly influenced by systemic cytokine activation and genetic or environmental factors that remain to be identified.

The interplay between barrier defects and immune abnormalities

In addition to its role as a physical barrier, the epidermis contributes to inflammatory responses. Keratinocytes (KCs) in AD are key producers of TSLP, which acts via OX40L on dDCs to polarize T cells toward a Th2 phenotype (Gittler *et al.*, 2013). Th2 cytokines IL-4, IL-13, and IL-31, along with Th22-derived IL-22, have been demonstrated to suppress terminal differentiation molecules (i.e., *FLG* and *LOR*), ultimately disrupting barrier function (Figure 1a). These findings led to the “inside-out” hypothesis of AD, suggesting that the epidermal changes are reactions to Th2 and Th22 signaling (Guttman-Yassky *et al.*, 2011). Recent studies have shown that the barrier and

immune defects are interactive players in the pathogenesis of AD (Gittler *et al.*, 2013). Moreover, immune activation through epicutaneous antigen exposure is an important mechanism that perpetuates the inflammation and immune-driven epidermal changes that characterize AD. Understanding the mechanisms of immune sensitization to topical antigens in AD patients may help us understand the factors that participate in disease onset.

New insights into AD pathogenesis obtained through contact sensitization

Molecules such as DNCB and other haptens provide a useful model for epicutaneous allergen exposure through intact or disturbed skin. Newell *et al.* (2013) elegantly showed equivalent penetration of DNCB, an almost universally sensitizing epicutaneous allergen, in AD patients, regardless of *FLG* mutation status. Through sensitization with DNCB, they showed Th2 polarization and attenuated hypersensitivity reactions in nonlesional AD skin compared with skin from healthy volunteers. Their data show that the unique immune phenotype of AD patients is perpetuated by allergen challenge regardless of mutation status.

Several important questions remain unanswered about contact sensitization in atopic individuals. Although DNCB provides an excellent experimental model for studying contact hypersensitivity, it is not an allergen that leads to ACD in a clinical setting. Penetration of “true” allergens that typically affect allergic individuals, such as nickel and rubber accelerators, may be more influenced by barrier defects and a patient’s genetic background than DNCB. Although Newell *et al.* (2013) demonstrated that background immune abnormalities in AD skin contribute to the

distinct Th2 polarization upon DNCB challenge, their approach does not address whether this holds true for commonly encountered allergens. Furthermore, compared with the almost universal potential for sensitization of DNCB, clinically relevant allergens affect different individuals with varying degrees of severity, and therefore immune differences among AD patients might influence allergen reactivity. In addition, both ACD and ICD are more common in AD patients. Although ACD is a delayed-type hypersensitivity reaction relying on antigen presentation in sensitized individuals, it has been suggested that ICD (Figure 1b) is a prerequisite for ACD (Figure 1c) (Bonneville *et al.*, 2007). ICD, which occurs via activation of innate immunity by KCs upon exposure to toxic irritants, may decrease the threshold for generating ACD reactions. This threshold may be decreased further in AD patients with defective barriers, increasing overall rates of allergen sensitization. However, despite the increased prevalence of allergic responses in AD, the resulting immune reactions are attenuated in these patients as compared with controls. This hyporesponsiveness may possibly be explained by altered LC or dDC function or differences in T-cell subsets in AD patients compared with nonatopic individuals.

Although it remains unclear where the primary abnormality lies in skewing T cells toward a Th2 phenotype in AD, insight is provided by DNCB-induced Th2 polarization through nonlesional AD skin, which we previously characterized with barrier and immune defects. Collectively, these concepts suggest that increased antigen penetration and/or altered antigen-presenting cell function in nonlesional AD skin result in an initial Th2-polarized response that can amplify over time into clinically inflamed lesions. The finding of Newell *et al.* (2013) that ACD in the context of AD is immunologically distinct, showing a Th2 rather than the conventional Th1 polarization, highlights the central role of the Th2 pathway in disease pathogenesis. In fact, emerging studies targeting IL-4R in AD patients show promising initial results (Simpson, 2013), supporting the pathogenic role of

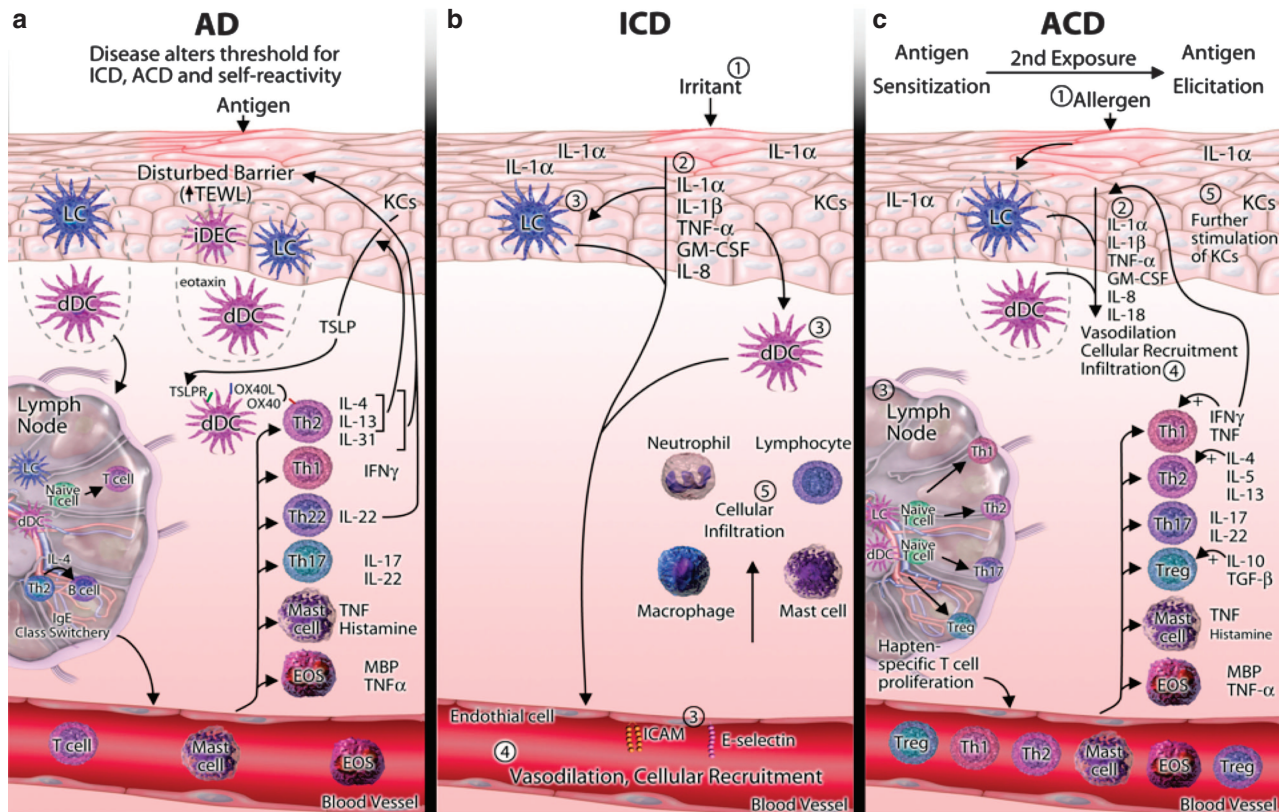


Figure 1. Immune mechanism in the pathogenesis of atopic dermatitis (AD), irritant contact dermatitis (ICD), and allergic contact dermatitis (ACD). (a) In patients with AD, a disturbed epidermal barrier leads to increased permeation of antigens, which encounter Langerhans cells (LCs), inflammatory dendritic epidermal cells (iDECs), and dermal dendritic cells (dDCs), activating T helper type 2 (Th2) T cells to produce IL-4 and IL-13. Dendritic cells (DCs) then travel to lymph nodes, where they activate effector T cells and induce IgE class switching. IL-4 and IL-13 stimulate keratinocytes (KCs) to produce thymic stromal lymphopoietin (TSLP). TSLP activates OX40 ligand-expressing dDCs to induce inflammatory Th2 T cells. Cytokines and chemokines, such as IL-4, IL-5, IL-13, eotaxins, CCL17, CCL18, and CCL22, produced by Th2 T cells and DCs stimulate skin infiltration by DCs, mast cells, and eosinophils (EOS). Th2 and Th22 T cells predominate in patients with AD, but Th1 and Th17 T cells also contribute to its pathogenesis. The Th2 and Th22 cytokines (IL-4/IL-13 and IL-22, respectively) were shown to inhibit terminal differentiation and contribute to the barrier defect in patients with AD. Thus, both the barrier defects and immune activation alter the threshold for ICD, ACD, and self-reactivity in patients with AD. TEWL, transepidermal water loss. (b) In patients with ICD, exposure to an irritant exerts toxic effects on KCs, activating innate immunity with the release of IL-1 α , IL-1 β , tumor necrosis factor (TNF)- α , GM-CSF, and IL-8 from epidermal KCs. In turn, these cytokines activate LCs, dDCs, and endothelial cells, all of which contribute to cellular recruitment to the site of KC damage. Infiltrating cells include neutrophils, lymphocytes, macrophages, and mast cells, which further promote an inflammatory cascade. (c) In the sensitization phase of ACD, similar to ICD, allergens activate innate immunity through KC release of IL-1 α , IL-1 β , TNF- α , GM-CSF, IL-8, and IL-18, inducing vasodilation, cellular recruitment, and infiltration. LCs and dDCs encounter the allergen and migrate to the draining lymph nodes, where they activate hapten-specific T cells, which include Th1, Th2, Th17, and regulatory T (Treg) cells. These T cells proliferate and enter the circulation and site of initial exposure, along with mast cells and EOS. On re-encountering the allergen, the elicitation phase occurs, in which the hapten-specific T cells, along with other inflammatory cells, enter the site of exposure and, through release of cytokines and consequent stimulation of KCs, induce an inflammatory cascade. MBP, major basic protein; TGF- β , transforming growth factor- β . Reprinted from Gittler *et al.* (2013), with permission from Elsevier.

Th2. Future studies are needed to address the role of allergic sensitization to common allergens in programming the AD immune phenotype.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

ND was supported by grant 5UL1RR024143-02 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and NIH Roadmap for Medical Research. NG was supported by NIH MSTP grant

GM07739. EG-Y was supported by the Dermatology Foundation Physician Scientist Career Development Award.

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Glucocorticoids in Autoimmune Bullous Diseases: Are Neutrophils the Key Cellular Target?

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The newly observed nongenomic effects of glucocorticoids on signaling pathways operating in neutrophils *in vitro* will stimulate further investigations on pathogenic mechanisms and drug actions in bullous skin diseases.

Journal of Investigative Dermatology (2013) 133, 2314–2315. doi:10.1038/jid.2013.205

There is by far no other drug class that is used so frequently by dermatologists as glucocorticoids. Their use as either topical or systemic anti-inflammatory drugs is based on their ability to block the expression of proinflammatory genes and to induce the transcription of anti-inflammatory genes (Rhen and Cidlowski, 2005). Besides these transcription-dependent mechanisms, glucocorticoids can have rapid effects on inflammation that are not mediated by changes in gene expression. For instance, the inhibition of vasodilation and reduction in endothelial permeability are believed to contribute to the anti-inflammatory activity of glucocorticoids (Rhen and Cidlowski, 2005). Other nontranscriptional mechanisms may include decreased stability of mRNAs of cytokine genes (Gille *et al.*, 2001). Furthermore, the activation of caspases by glucocorticoids in eosinophils, but not neutrophils, has been described (Meagher *et al.*, 1996). There is, however, little information regarding

the molecular mechanisms of these so-called nongenomic pathways available.

Bullous pemphigoid (BP) represents the most frequent autoimmune subepidermal blistering disease, characteristically associated with autoantibodies directed against BP180 (also termed BPAG2) and BP230 (BPAG1-e), two components of junctional adhesion complexes called hemidesmosomes (Borradori and Sonnenberg, 1999). Besides these autoantibodies, neutrophils have been shown to have a critical role in BP (Liu *et al.*, 1997). In this issue of the *Journal*, Hellberg *et al.* (2013) investigated the effects of methylprednisolone on human neutrophils as BP can be efficiently treated with either topical or systemic glucocorticoids (Joly *et al.*, 2002). The authors hypothesized that methylprednisolone might directly block neutrophil activation here (Figure 1). Intriguingly, they observed in fact a marked block of autoantibody-induced activation of phosphatidylinositol

3-kinase (PI3K), p38 mitogen-activated protein kinase (MAPK), and extracellular signal-regulated kinases 1/2 (ERK1/2) in purified human neutrophils *in vitro*. As these findings are provocative, the authors have additionally demonstrated that methylprednisolone, as well as more or less specific inhibition of these kinases by known pharmacological inhibitors, could block the production of reactive oxygen species (ROS) in activated neutrophils. Methylprednisolone and the kinase inhibitors were also able to prevent blister formation in both *ex vivo* and *in vivo* BP models.

The activation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, the enzyme largely responsible for ROS production in neutrophils, has previously been shown to require activation of pathways involving p38 and PI3K (Geering *et al.*, 2011). If methylprednisolone is indeed able to pharmacologically inactivate these kinases, under the *in vitro* conditions used by the authors, it is not surprising that it also blocks ROS production by activated neutrophils. It is, however, less clear that neutrophil-derived ROS are required for blister formation in BP. Moreover, it should be noted that these authors used whole leukocyte populations for their *ex vivo* model. Therefore, it is unclear whether the observed epidermal–dermal separation was indeed mediated selectively by neutrophils. The situation *in vivo* is even more complex; it is uncertain whether a potential inhibition of PI3K, p38 MAPK, or ERK1/2 in neutrophils is indeed the main effect responsible for the prevention of blister formation. In addition, it should be noted that with these *in vivo* models all inhibitors were given in a prophylactic and not in a therapeutic manner, suggesting that the initiation of the inflammatory cascade was blocked, including neutrophil infiltration. Similarly, the question whether methylprednisolone could reduce kinase activation of already-activated neutrophils remains to be answered.

Besides these fundamental questions, which need to be answered in future studies, an additional note of caution ought to be raised. The authors used extremely high methylprednisolone

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