variant as damaging to normal PLCδ1 function. Dysregulated calcium signaling that ensues may be implicated in cyst formation, as seen in renal cysts (Kuo et al., 2014).

A monoallelic "two-hit" mechanism leads to trichilemmal cyst formation

To date, dominant truncating and recessive missense germline variants in *PLCD1* have been identified in patients with familial leukonychia (Kiuru et al., 2011). In this study by Hörer et al. (2019), a dominantly inherited haplotype with a synonymous and missense variant seen in tandem constitutes a "high-risk allele", and this genetic difference may account for the lack of leukonychia in these patients. This allele predisposes to the acquisition of in cis somatic variants within the C2 domain of PLCδ1, which is associated with cyst formation. It still is unclear as to why the high-risk allele predisposes to somatic acquisition of the cyst-specific variant, however, this is a recognized finding in other genes such as *JAK2*, where "hypermutable" and "fertile-ground" hypotheses have been posited as hypothetical models to explain how germline variants may attract additional somatic mutation events (Campbell, 2009).

The genetic study of cyst material has been central to the discovery of the proposed monoallelic model. Because of the nature of the techniques employed in this study, it remains to be proven if there is loss of heterozygosity involving the cyst-specific variant, and this could be investigated using single cell sequencing approaches in future studies. To date, Knudson's two-hit hypothesis has been frequently demonstrated in a range of skin tumor predisposition syndromes, as well as in cyst formation in other tissues, such as the kidney. Recently, however, evidence for monoallelic mechanisms have been emerging in renal cyst formation (Cornec-Le Gall et al., 2018), adding support to the proposed mechanism here in the skin. Cyst formation has been associated with loss of planar cell polarity and deregulated primary ciliary function, and it would be of interest to determine PLCδ1's role in these important tissue-patterning mechanisms.

This work by Hörer et al. (2019) is important as it highlights PLCδ1 as an important protein in the maintenance of human hair follicle homeostasis. In addition, the *PLCD1* "high-risk" allele identified may inform the interpretation of genetic tests in families with trichilemmal cysts. Finally, the monoallelic model of cyst formation proposed may be relevant in other genodermatoses and highlights the importance of studying genetic changes in the skin in conjunction with peripheral leukocyte DNA.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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ILC2s and Basophils Team Up to Orchestrate IL-33–Induced Atopic Dermatitis

Bernhard Ryffel[1] and José Carlos Alves-Filho[2]

In this issue, Imai et al. (2019) provide new insights into the pathophysiology of AD-like inflammation using their model (Imai et al., 2013) and ask how ILC2s and basophils contribute to the IL-33–induced AD-like inflammation. Their findings show that continuous expression of IL-33 in keratinocytes is sufficient to cause AD-like inflammation in mice, and that this occurrence is largely independent of adaptive immune cells and is mediated by basophils and ILC2s.

Journal of Investigative Dermatology (2019) **139,** 2077–2079. doi:10.1016/j.jid.2019.06.118

Atopic dermatitis (AD) is an important clinical disease with different, often severe, manifestations. Causative agents may include chemicals of environmental or plant origin; genetic predisposition factors are also relevant

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Clinical Implications

- IL-33–driven AD is independent of adaptive immunity.
- Keratinocyte-derived IL-33 activates basophils, which induce ILC2s.
- Basophil depletion in this context is of potential therapeutic interest.

(Weidinger and Novak, 2016). Mechanistic insights may be obtained from mouse models (Nakajima et al., 2019). Experimentally, AD can be induced by the topical application of proteins, chemicals, inflammatory cytokines, histamines, and other mediators that distort the epithelial immune microenvironment (Dainichi et al., 2018). IL-33 is a pleiotropic cytokine playing a pivotal role in allergic disorders, including AD (Liew et al., 2016). Intradermal injection of IL-33 in the skin caused psoriasis-like neutrophilic inflammation with mast cell activation in mice (Hueber et al., 2011). Topical application of calcipotriol (a vitamin D3 analogue) induces expression of thymic stromal lymphopoietin (Li et al., 2006) and causes IL-33–dependent AD-like inflammation involving dendritic cell activation with a distinct type 2 immune response (Li et al., 2017).

Imai et al. (2013) established a transgenic mouse expressing IL-33 in keratinocytes driven by the keratin-14 promoter (IL33tg) that is characterized by epidermal hyperkeratosis as well as eosinophil and innate lymphoid cell type 2 (ILC2) infiltration. Now, the authors provide new insights into the pathophysiology of AD-like inflammation using their model (Imai et al., 2013) and ask how ILC2s and basophils contribute to the IL-33–induced AD-like inflammation (Imai et al., 2019). Skin inflammation developed in B cell– and T cell–deficient Rag2KO IL33tg mice suggests that adaptive immune cells are dispensable. In contrast, the depletion of ILC2s accomplished via bone marrow transplantation from IL33tg to ILC2-lacking, RORα-deficient mice suppressed the development of AD-like inflammation. The authors then explored the role of the basophils in IL33tg skin inflammation. Specific depletion of basophils with the anti-FcεRIα antibody MAR1 (Sokol et al., 2008), or conditional diphtheria toxin–induced basophil depletion using Bas-TRECK mice (Sawaguchi et al., 2012) crossed with the IL33tg mice, reduced AD-like inflammation. These results provide strong support for a critical role of basophils in this model, consistent with a previous report (Nakashima et al., 2018). Moreover, the authors demonstrated that depletion of basophils in IL33tg skin reduces the accumulation of ILC2s and ILC2-derived cytokines and chemokines, including IL-5, IL-13, and CCL5. Therefore, the data suggest that keratinocyte-driven, IL-33–mediated AD-like inflammation may be initially and solely dependent on an innate immune response mediated by ILC2s acting in concert with basophils (Figure 1).

The study is of interest because it shows that continuous expression of IL-33 in keratinocytes is sufficient to cause AD-like inflammation in mice, and that this occurrence is largely independent of adaptive immune cells and is mediated by basophils and ILC2s. ILC2s have emerged as important participants in early allergic responses in skin that pave the way for T helper type 2 cell responses. However, the observation that basophils are required for the ILC2 differentiation is novel and thus may open new therapeutic considerations.

There are limitations of this model, because the mechanisms explored involve IL-33 production that is restricted to keratinocytes. AD in patients clearly depends on other inflammatory factors and cell types. In addition, RORα-dependent ILC2 depletion may affect cytokine production from group 3 innate lymphoid cells and T helper type 17 cells (Lo et al., 2016). The roles of basophils and ILC2s should be investigated additionally in other experimental models of AD and in patients.

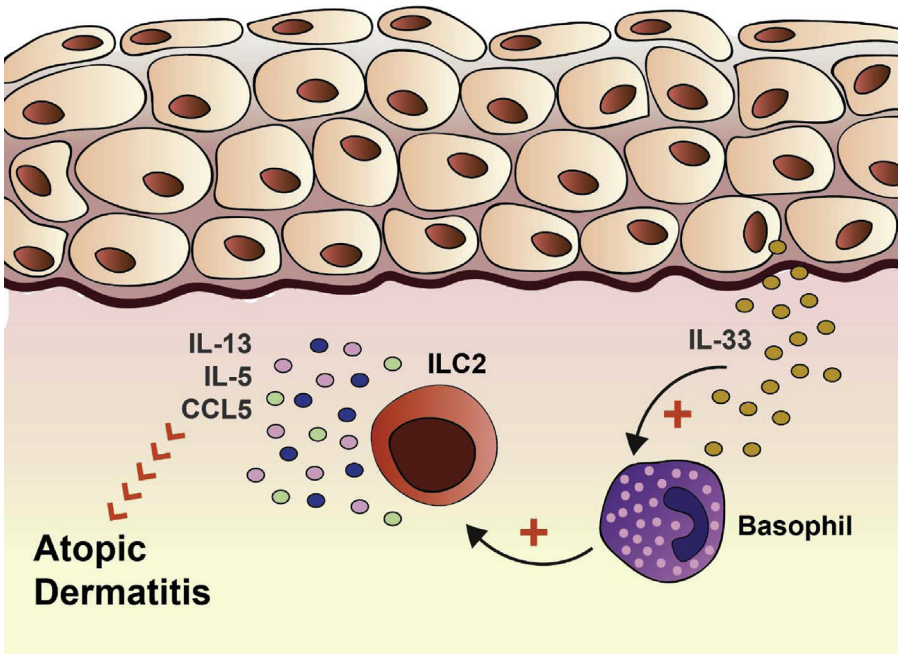

Figure 1. ILC2s and basophils team up to orchestrate IL-33–induced atopic dermatitis. Transgenic IL-33 expression in keratinocytes activates basophils, which recruit ILC2s producing IL-5, IL-13, and CCL5, driving initially AD-like skin inflammation independent of adaptive immunity. AD, atopic dermatitis; ILC2, innate lymphoid cell type 2.

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