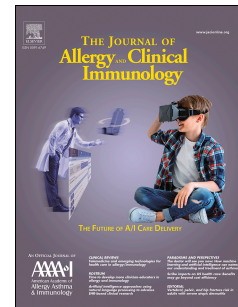


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Molecular Mechanism-driven New Biomarkers and Therapies for Atopic Dermatitis

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The paper by Hong, JT and associates in this issue of JACI focuses on the role of IL-32 γ , an isotype of IL-32, in the development of atopic dermatitis (AD), an inflammatory allergic skin condition that primarily occurs in the bends of the neck, face, arms, and legs, affecting ~10% of the global population [1,2]. AD is characterized by raised, irregularly shaped, red, and intensely itchy lesions that can result in epidermal thickening, hyperkeratosis, parakeratosis, and acanthosis. Diagnosis is based on these signs and symptoms, with current treatments utilizing immunosuppression at the local sites of inflammation by the application of steroid creams, or oral prednisone for severe cases. Apart from drug treatment, significant lifestyle changes can be a part of an AD treatment plan, including the avoidance of substances that are found to trigger or aggravate the presenting symptoms [2]. The precise cellular and molecular mechanisms of AD remain unclear, however, the disease is thought to result from a combination of factors, such as genetics, environmental stimuli, skin permeability, and immune system dysfunction.

Significant cellular mediators of AD pathogenesis include T cells, Mast cells, group 2 Innate lymphoid cells (ILCs), and keratinocytes. The cellular mechanisms underlying AD pathogenesis are schematically shown in Figure 1A. AD onset in children is TH2-dominated and characterized by short-term activation, whereas adult AD extends to additional TH subsets, including TH1 and TH22 [3]. Further, human primary keratinocytes produce multiple inflammatory cytokines including IL-32, which contributes to both the infiltration of pro-inflammatory cells and the process of allergic inflammation [4]. Apoptosis of keratinocytes in response to IFN- γ and TNF- α signaling is a major driver of AD pathogenesis. Up regulation of IL-32 in keratinocytes has not only been shown to enhance this apoptosis but also to be sufficient to trigger apoptosis of keratinocytes on its own. Further, the knockdown of IL-32 in

keratinocytes decreases their apoptosis [4]. Apart from allergic inflammation, IL-32 is also involved in the inflammatory processes of rheumatoid arthritis, chronic obstructive pulmonary disease, and Crohn's disease. Previously, IL-32 α , a shorter splice variant of IL-32 was shown to suppress endothelial cell inflammation by inhibiting miR-205 expression, which is regulated by NF- κ B as a downstream target of RELA (p65) [5].

In this issue of JACI, Hong JT and associates report a novel role of IL-32 γ , a full-length active isoform of IL32 in AD and describe the molecular mechanism of AD pathogenesis [1]. The authors hypothesized that IL-32 γ expression suppresses NF- κ B activation leading to decreased expression of miR-205, which in turn decreases the development of T cell-mediated severe AD pathology [1]. To explore the relationship between IL-32 γ and AD, authors induced AD pathology in male IL-32 γ transgenic (tg) mice using phthalic anhydride (PA) or MC903 (a vitamin D3 analog). The results demonstrated that PA-induced IL-32 γ mice had reduced AD pathology along with a reduction in the number of infiltrating inflammatory cells. Further, the authors demonstrated that IL-32 γ was able to prevent translocation of the NF- κ B family transcription factors p50 and p65, as well as activation of I κ -B α in PA-induced skin tissues. In addition, IL-32 γ treatment was shown to increase miR-205 expression using a qPCR assay, suggesting a direct relationship between IL-32 γ and miR-205. However, this warrants further *in-vitro* and *in vivo* studies.

Further, treatment of the AD mouse model with human IL-32 γ reduced expression of inflammatory cytokine genes, such as IFN- γ and TNF- α ; and hence AD inflammation. Analysis of IL-32 γ , IL-33 and miR-205 levels in human serum by ELISA showed that each of these was significantly increased in AD patients compared to those in healthy controls, suggesting a

clinical correlation of IL-32 γ , not IL-31(control) and miR-205 in human AD. This provides good preliminary clinical evidence for human AD compared to what was observed experimentally in the mouse models. These novel findings on the role of IL-32 γ and AD both experimentally and clinically present evidence for pathological reduction in AD in the presence of IL-32 γ and warrant further investigation by other groups prior to possible therapeutic development.

It is to be emphasized that despite progress made, our knowledge of molecular genetic mechanisms underlying severe chronic AD is fairly limited. It has been shown that loss of function mutations in the FLG gene encoding filaggrin is a major risk factor for AD development [6]. Filaggrin facilitates proper skin barrier formation, and defects in the protein can result in moisture escape and microbial incursion. This lends rationale as to why individuals with AD often have dry skin that is prone to infection. Also, novel heterozygous mutations in CARD11 were observed to have a link to AD due to the alteration of a scaffolding protein of a lymphocyte receptor [7]. Mutations in CARD11 resulted in diminished IFN γ cytokine production, NF- κ B and mTORC1 activation. Of note, all participants in this study that exhibited CARD11 mutation presented with AD, and restoration of the normal CARD11 gene reduced inflammation in laboratory models. The CARD11 gene converges on NF- κ B and IFN γ by way of cytokine signaling (Figure 1A), which regulates inflammatory IL-32 γ induction.

To further map the molecular pathways and networks known to be associated with AD, we conducted an Ingenuity Pathway Analysis (IPA) (QIAGEN Inc.) of the GEO RNA expression database (series GSE140227 and series GSE 140380) using the search term "atopic dermatitis" (8). Of a total of 412 genes/proteins known to play a role in AD, 141 are

found to also be involved in IFNG/NF κ B/IL-32 signaling (Figure 1B). These pathways when overlaid with expression data from the GEO database that compares normal human skin vs AD samples, lead to a network plot of the genes/proteins with altered expression in AD and direct connection to IFN γ , IL32, Mir-205, and NF κ B. These pathway analyses and the results of Hong et al suggest major regulators of AD as new biomarkers for prognosis and therapeutic targets.

Finally, AD has been shown to be a co-morbidity for hay fever and asthma in many cases [9]. Individuals that have either of these respiratory allergic conditions, or a family history, have a greater preponderance for developing AD. IL-32 γ was recently shown to suppress the development of an allergic airway phenotype and reduce incidence of asthma [10]. This method may also have other benefits outside of treating AD and asthma and should serve as an additional factor that warrants greater efforts in translational application. Collectively, this work shows a pivotal role for IL-32 γ in reducing AD-associated inflammation and could very well pave the way for future therapeutics, and additional scientific discovery into AD.

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Conflict of interests. The authors declare that they have no competing interest.

Figure 1. A) A schematic of cellular and molecular mechanisms underlying acute and chronic AD. Keratinocyte activation in response pathogen/allergen produces IL-32 γ , initiates the cross-talk with Langerhans cells (LC) and activates TH2 response. Whereas the acute AD involves dominant TH2 response, the chronic AD becomes more TH1 dominant response. B) The results of IPA analysis showing novel regulatory molecular mechanisms active in this network during chronic AD. All known interactors (direct and indirect) with Mir-205 and CARD11 are displayed and then checked for differential expression between the normal and AD samples. The symbols express gradual changes in expression where Red= increased, green=decreased and grey= no or unknown expression.

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