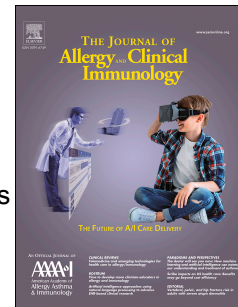


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Advances in Atopic Dermatitis in 2019-2020: Endotypes from skin barrier, ethnicity, properties of antigen, cytokine profiles, microbiome, and engagement of immune cells

Running title: Emerging chart of AD endotypes

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Abstract

Key research advances in atopic dermatitis (AD) suggest the complexity of its endotypes. A comprehensive serum biomarker panel revealed at least four types of AD. Some represent classic TH2-dominant AD with filaggrin mutations commonly reported in Europeans, a simultaneously activated multipolar axes of cytokines often reported in Asians, and an intrinsic type characterized by TH2-inferiority. Innate lymphoid cells, including NK cells, NKT cells, and fibroblasts, play a role in AD development and heterogeneity. Here, we discuss the endotypes of AD from the perspective of antigen types (hapten vs. protein antigens), barrier function, and a novel set of immune cells. Endotypic stratification of AD may lead to the development of customized therapeutic strategies in the future.

Keywords: atopic dermatitis; endotypes; haptens; protein antigens; ethnic differences; innate immunity; acquired immunity

Abbreviations atopic dermatitis (AD); interleukin (IL)-4 receptor alpha (IL-4R α); once weekly (qw), every 2 weeks (q2w); severity scoring of atopic dermatitis (SCORAD); eczema area severity index (EASI); dendritic cells (DCs); innate lymphoid cells (ILCs); 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD); 6-formylindolo[3,2-b]carbazole (FICZ); Indolo-[3,2-b]-carbazole (ICZ); 3,3'-diindolylmethane (DIM); indole-3-carbinal (I3C); indole-3-aldehyde (IAld); *S aureus* second immunoglobulin-binding protein (Sbi); dermal dendritic cells (dermal DCs); periostin (POSTN)

1. Introduction – novel treatment targets and emerging complexity of AD

Atopic dermatitis (AD) is one of the very common inflammatory skin diseases.¹⁻⁹ Historical precursors of atopic dermatitis (AD) date back to a description of Emperor Augustus documented by Roman historian Suetonius (A.D. 69-140).¹⁰ Fred Wise (1881-1950) and Marion Sulzberger (1895-1983) introduced the term atopic dermatitis to dermatology in 1933.¹⁰ This definition was based on atopy, a term coined by Arthur Coca (1875-1959) and Robert Cooke (1880-1960) in 1923, when they recognized an association between allergic rhinitis and asthma.¹⁰ Understanding of AD has progressed with the discovery of IgE and type 2 immune responses and has culminated in the introduction of dupilumab, a humanized anti-human interleukin (IL)-4 receptor alpha (IL-4R α) monoclonal antibody.¹¹ Further efforts is continued to target specific cytokines and signaling pathways, such as IL-13, IL-22, IL-31 and its receptor A, IL-32 γ , IL-33, OX40, Janus kinase inhibitors, SYK inhibitors, phosphodiesterase 4, miR-205-mediated regulation of nuclear factor- κ B (NF- κ B), miR-335-mediated regulation of SOX6, histamine H4 receptor, and mammalian target of rapamycin complex 2.¹²⁻²⁴

The clinical course and responsiveness of patients show variation, indicating heterogeneity in the mechanism of AD.^{8, 9, 25} Such variation reflects the heterogeneity of AD in terms of age, chronicity of disease, ethnicity, race, genetic background (presence or absence of filaggrin mutation), barrier function, antigen properties (hapten and protein), characteristics of engaged immune cells of innate and acquired immunity, source of pruritus (IL-4, IL-13, IL-31, thymus and activation-regulated chemokine [TARC]/C-C motif chemokine ligand 17 [CCL17], and thymus stromal lymphopoietin [TLSP]), cytokines (type 2-helper T cell [TH2], TH17, and TH22 cytokines, and IL-1 β), and metabolic status such as obesity.^{8, 9, 25-27} Scaffold protein such as caspase recruitment domain family member (CARD) 11 and CARD14 are involved in AD-like dermatitis.²⁸⁻³⁰ Itching and skin barrier defect contribute to the onset of AD as well.^{17, 31-}

⁴¹ Here, we have reviewed the evidence that indicates AD endotypes and have discussed the underlying mechanisms that define these endotypes. Subtitles in each section indicate the new findings reported in *the Journal of Allergy and Clinical Immunology* between 2019 and 2020.

2. Modifiers of cytokine axes – roles of TSLP and PGE₂

Various factors affect cytokine axes. A single application of hapten to sensitized individuals induces TH1-dominant dermatitis in the form of contact hypersensitivity (CHS) in mice (a model for human contact dermatitis).^{42, 43} Repeated applications of hapten alter this major cytokine axis to TH2, causing AD-like inflammation in mice, such as that mediated by IL-4 production in the draining lymph nodes and hapten-specific IgG1 and IgE induction.^{42, 43} This is consistent with the concept of the "hapten-atopy hypothesis," as discussed below.^{44, 45}

IL-17A deficiency suppresses repeated hapten application-induced murine dermatitis.⁴⁶ Consistently, IL-17A deficiency attenuates spontaneous development of AD-like dermatitis and IgE induction in flaky-tail mice (*Flg^{fl/fl} mal/mal*).⁴⁶ The major source of murine IL-17A is V γ 4⁺ $\gamma\delta$ T cells in the skin-draining lymph nodes and V γ 5⁺ dermal $\gamma\delta$ T cells.⁴⁶ These cells may produce IL-17A in human AD, especially in the TH17-axis-activated subgroup of Asian AD, which warrants further study.

Dectin-2 is another factor that presents an association between the TH2 and TH17 axes.⁴⁷ TSLP catalyzes the activation of phosphorylated signal transducer and activator of transcription 5 (pSTAT5) and aids the recruitment of TET2 (a protein converting 5-methylcytosine to 5-hydroxymethylcytosine to induce DNA demethylation) to *FCER1G* (the high-affinity IgE receptor γ subunit gene). This leads to *FCER1G* demethylation and upregulation of expression of FcR γ -related receptors on human monocytes.⁴⁷ Monocyte-derived DCs in the presence of TSLP help maintain upregulation of expression of FcR γ -related receptors and support TH2/TH17 polarization in a dectin-2 dependent way *in vitro*.⁴⁷ Such a mechanism may help explain the co-activation of the TH2 and TH17 axes in a subgroup of AD.

Prostaglandin E2 (PGE₂) also affects AD as a negative regulator of dermatitis.^{48, 49} Prostanoids are produced in AD lesions in addition to the keratinocyte-derived cytokines, such as TSLP and IL-33.⁴⁸ Their functions were investigated in a mouse model of AD.⁴⁸ PGE₂ activity decreased TSLP levels by downregulating the surface expression of protease-activated receptor 2 (PAR-2) via the receptor for PGE₂ known

as EP2.⁴⁸ A complete understanding of the PGE₂ role in human AD warrants further study.

These lines of evidence suggest that subtypes of AD are generated by different contributions of cytokines, chemokines, and autacoids, including prostaglandins.

3. Heterogeneous cytokine profiles among AD patients – differences such as IL-22 level among races

The efficacy of dupilumab has affirmed AD as an IL-4/IL-13-driven condition in Caucasians, Asians, and African Americans.⁵⁰ A study analyzed datasets of two phase 3 trials with identical designs, namely LIBERTY AD SOLO 1 (NCT02277743) and LIBERTY AD SOLO 2 (NCT02277769), consisting of two 16-week phase 3 studies in adults with moderate-to-severe AD (n = 1,379) inadequately controlled with or contraindicated for topical medications, randomized to dupilumab 300 mg once weekly (qw), every 2 weeks (q2w), or placebo.⁵¹ Dupilumab helped improve symptoms over time versus marginal changes with placebo.⁵¹ Treatment with dupilumab qw, q2w, or placebo helped achieve improvement from baseline of ≥50% in eczema area and severity index (EASI-50) in 61%, 67%, or 23% of the participants.⁵¹ This result implied that 39% or 34% of the patients with qw- or q2w-dupilumab failed to achieve EASI-50 at week 16, respectively. Therefore, the pathogenesis of AD remains unexplained with sole consideration of IL-4/IL-13 signaling.^{9, 52}

Heterogeneity of AD has been reported among different racial groups according to clinical, molecular, and genetic differences (Table 1).^{9, 53-69} The healthy skin of people of Caucasian, African, or Asian descent differs in epidermal gene expression, such as *NINL* (*Homo sapiens* ninein-like) and *S100A4* (S100 calcium-binding protein A4).⁷⁰ Such variation may affect the onset or features of AD.^{54, 55, 71, 72} European American, African American, and Asian AD cases present a common TH2-dominant cytokine profile, while additional cytokine axes are differentially activated in these subgroups.^{54, 55, 63, 71, 72}

European American AD is characterized by a TH2-centered cytokine profile with a TH2 component with attenuated TH17 (Table 1).^{9, 53-65} The intralesional profiling of acute and chronic dermatitis presents with differences.⁶⁵ Acute lesions exhibit increased levels of terminal differentiation protein (S100As), TH2/TH22-cytokines (IL-4, IL-13, IL-31, and IL-22), and lesser TH1-cytokines (IFN- γ , MX1, IL-1 β , and CXCL9/CXCL10/CXCL11), while chronic lesions exhibit strengthened expression of TH2-cytokines, elevated levels of K16 and Ki67 (keratinocyte proliferation), increased expression of TH1-cytokines, IL-22, and its associated products such as S100As and IL-32.⁶⁵

Asian AD cases present with expansion of IL-17⁺ T cells in the skin and blood (Table 1).^{9, 53-65} The Asian AD endotype is characterized by stronger induction of markers for TH17 (IL-17A, IL-19, CCL20), TH17/TH22 (S100A12), and TH22 (IL-22), along with lower TH1 activation in comparison with European Americans.⁵⁶ TH2 induction (IL-13, CCL17, CCL18, CCL22) is similar between Asian and European American groups.⁷¹ Serum analysis results indicate a TH2/TH22 profile with lower TH1/IFN- γ .^{71, 72} Genomic profiling reveals that Asian endotypes demonstrate a mixture of immune dysregulation and barrier dysfunctions.^{55, 56} Skin samples obtained from Japanese and Korean patients with AD show changes in epidermal thickness (psoriasiform phenotype). Han Chinese with AD exhibit a similar phenotype to Japanese and Korean AD, and this phenotype is characterized by increased epidermal hyperplasia, parakeratosis, and mRNAs for TH2 (*IL4*, *IL4R*, *IL13*, *IL5*, *IL10*, *IL31*, *CCL13/17/18/22/26*), TH17/IL-23 (*IL17F*, *IL19*, *IL21*, *CCL20*, *PI3* [peptidase inhibitor 3], *DEFB4* [defensin β 4]), and TH17/TH22 (*IL22*, *S100As*).⁵⁵ Severity scoring of atopic dermatitis (SCORAD) correlates with IL-31, IL-5, and IL-17A expression in Han Chinese, wherein the Chinese AD phenotype is characterized by increased TH17 expression and lower levels of TH1/IFN- γ -related markers.⁵⁵ Accordingly, epidermal changes occurring in Asian AD patients include increased hyperplastic (thickness and Ki67 counts), parakeratotic, and focal hypogranulosis.⁵⁶ However, the barrier protein loricrin (LOR) and filaggrin (FLG) are maintained, unlike the features reported in European Americans.⁵⁶ These findings suggest different etiologies of AD among races, wherein epidermal differentiation abnormalities are more significant in European American patients.

African American AD is a condition elucidated via global molecular profiling (Table 1).^{9, 53-65, 73} The magnitude of TH2/TH22 upregulation (CCL17/18/26, IL-22, and S100As) is similar between African American and European American AD patients.⁵⁴ A few TH2 markers (TSLP, IL-10, and CCL13) present with more upregulated expression in African American AD compared to that reported in patients of European descent.⁵⁴ Conversely, African American patients exhibit lower expression of innate immunity markers (TNF, IL-1 β), TH1 (IFN- γ , MX1, and IL-12R β 1), and TH17 (IL-23p19/IL-23A, IL-36 γ , and CXCL1).⁵⁴ Such TH2-dominancy in African Americans is underlined with (1) increased infiltration of dendritic cells (DCs) with the high-affinity IgE receptor in comparison with European American patients and (2) higher serum IgE levels in the African American cohort.⁵⁴ SCORAD correlates positively with serum IgE in the African American AD group; however, such a correlation is not observed in the European American group.⁵⁴ In terms of barrier proteins, African American patients present with downregulation of loricrin (LOR) expression but not filaggrin (FLG).⁵⁴

A post hoc study of dupilumab indicates different responses among European, African and Asian descendants.⁷⁴ Three phase 3 trials (LIBERTY AD SOLO 1 (NCT022777743), SOLO 2 (NCT022777769), and CHRONOS (NCT02260986)) include 2,058 patients comprised of White (n = 1,429), Asian (n = 501), and Black/African American (n = 128). Measured outcomes in this study were change from baseline to week 16 eczema area severity index (EASI), weekly peak pruritus numerical rating scale (NRS), dermatology life quality index (DLQI), patient-oriented eczema measure (POEM), investigator's global assessment (IGA), and pain or discomfort assessed by the European quality of life-5 dimensions 3 level questions (EQ-5D-3L). Dupilumab was effective in all racial subgroups as expected. In White and Asian subgroups, both dupilumab 300 mg q2w and qw improved signs for atopic dermatitis (EASI, IGA), symptoms (POEM) including itch (peak pruritus NRS) and pain and discomfort (EQ-5D-3L), and quality of life (DLQI) at week 16. In Black/African American patients both regimens led to improvement in the EASI and mean change in peak pruritus NRS, while only dupilumab 300 mg qw showed improvement in mean percent change in peak pruritus NRS, POEM, and proportions of patients achieving the IGA success. Weight-adjusted analyses were consistent with these findings. This finding again suggests the presence of unappreciated heterogeneity

in the underlying mechanism for each symptom of AD.

In summary, European descendants demonstrate defects in LOR and FLG with a dominance of TH2/TH22. Asians tend to experience additional activation of TH1/TH17-cytokine axes with less involvement of LOR and FLG. Africans present with less involvement of TH1/TH17 with barrier defects only in the LOR.

4. AD clusters defined by serum biomarkers – IL-32 γ and miR-205

Biomarkers are keystones to understand the mechanism of AD and establishing new treatments.⁷⁵ IL31, IL-32 γ (an isotype of inflammatory cytokine IL-32), IL-33, and miR-205 microRNA are increased in serum of AD patients.²³ IL-32 γ inhibits the expression of miR-205 microRNA probably via an inhibition of NF- κ B activation.²³ As IL-32 γ suppresses AD-like dermatitis in mouse models, it can be a therapeutic target as well.²³

Analysis of 143 serum biomarkers obtained from 146 patients with severe AD (median EASI 28) has helped identify four clusters (Figure 1) recently.⁷⁶ Cluster A (34%, median EASI 28) distinguishes a skin-homing chemokine/IL-1R1-dominant cluster. Cluster B (18%, median EASI 25) is TH1/TH2/TH17-dominant. Cluster C (18%, median EASI 38) is TH2/TH22/PARC-dominant. Cluster D (30%, median EASI 27) is TH2/eosinophil-inferior. This result confirms the immunological heterogeneity of AD. Cluster A is distinct from others because of higher levels of C-C chemokines (cutaneous T cell-attracting chemokine [CTACK]/CCL27, thymus, and TARC/CCL17, macrophage-derived chemokine [MDC]/CCL22, and regulated upon activation, normal T cell expressed and presumably secreted [RANTES]/CCL5) and IL-1R1. Cluster A comprised a higher proportion of patients treated with systemic immunosuppressants (excluding systemic corticosteroids) within a year before sampling (37% in cluster A vs. 18% in cluster B, 11% in cluster C, and 16% in cluster D). The effect of these immunosuppressants on the formation of cluster A is unknown. Cluster B is characterized by an inflammatory state with the highest levels of TH2-related cytokines (IL-4, IL-5, and IL-13), TH1-related (IFN- γ , TNF- α , and TNF- β), TH17 related (IL-17 and IL-21), and epithelial-related (IL-25,

IL-33, and TSLP) cytokines. TH1/TH2/TH17-dominance of cluster B is similar to that observed in East Asians with AD.^{9, 56} Cluster C is enriched in patients with higher severity scores (median EASI = 37.8) and high levels of TH2-related cytokines (PARC, IL-13, IL-5, eotaxins), IL-22, and IL-33. Cluster C may include patients with elevated IL-22 levels, a condition which can be ameliorated by considering conduction of fezakinumab-mediated IL-22 blockade.⁵² Cluster D exhibited a low inflammatory state with low serum levels of TH2/severity-related (MDC, PARC, and TARC) and eosinophil-related (RANTES, eotaxins) markers. The inferior features of TH2 cytokines and eosinophils are similar to the features of the intrinsic type of AD.^{7, 8, 27}

5. The mode of antigen invasion and antigen properties revisited – p-phenylenediamine (PPD)-mediated transcriptomic changes in human skin

The repeated application of hapten induces AD-like dermatitis in mice.^{25, 42, 43, 77-80} In this model, a shift from a TH1- to TH2-response occurs with elevated IL-4 expression, cutaneous infiltration of eosinophils, and elevated levels of hapten-specific serum IgE.⁴² This concept is proposed as the "hapten-atopy hypothesis".^{7, 44, 45} Consistently, contact sensitization is a frequent occurrence in patients with AD.⁸¹ The identity of cells that mediate the shift of local immunity from TH1 dominance to TH2 dominance is debated. Basophils are one such candidate that express MHC class II and IL-4 in the draining lymph nodes (Figure 2).⁸²

On skin barrier defects, macromolecules (proteins) permeate through the tight junction and are captured by Langerhans cells (Figure 2). Epicutaneous application of ovalbumin (OVA) elevates serum OVA-specific IgE and IgG1 levels in a TH2-dependent manner and induces dermal infiltration of T cells, eosinophils, and neutrophils, and increases the local expression of *IL4*, *IL5*, and *Ifng*.⁸³

Thus, bifurcation of inflammation type depends on the properties of antigens, such as the size and integrity of the skin barrier condition. This may help explain the mechanism

of intrinsic and extrinsic AD.^{7, 27} Molecular phenotypes of these types are described in European American patients (Table 1).^{27, 58} The extrinsic phenotype accounts for 80% of the AD cases with high total and environmental serum levels of IgE, eosinophilia, family history of atopic disease, and an increased rate of FLG mutation.^{27, 58} The intrinsic phenotype accounting for 20% exhibits normal IgE levels, female predominance, preserved barrier function based on transepidermal water loss, and increased contact hypersensitivity to metal; however, these patients usually lack an atopic background or FLG mutation.²⁷ TH2 induction is similar between these endotypes.^{27, 58} However, the intrinsic type represents increases in TH1 (IFN- γ , CXCL9/10, and MX1) and TH17/TH22 (IL-17A, CCL20, PI3, and IL-22) products together with S100A9/12 (regulated by IL-17 and IL-22).^{27, 58} Regarding SCORAD, TH1- and TH17-related cytokines positively correlate in intrinsic AD, whereas in extrinsic AD, only correlation with TH2 cytokines (IL-4 and IL-5) is noted.⁵⁸ Blood IFN- γ -producing TH1 level is increased in intrinsic type (IgE-low AD group).⁸⁴ These features of intrinsic and extrinsic endotypes match the experimental findings in mouse models.^{7, 27, 42, 43, 58, 83, 85}

The effect of p-phenylenediamine (PPD) on primed and unprimed human skin has been analyzed on a molecular level.⁸⁶ PPD is a strong contact allergen used in hair dye that is known to cause allergic contact dermatitis (ACD). Biopsied skin samples were collected from 4 patients with mild and 5 patients with severe PPD-related ACD and 7 hairdressers without contact dermatitis on day 4 after patch testing with 1% PPD in petrolatum.⁸⁶ RNA sequencing and transcriptomics analyses were performed and confirmed by using quantitative RT-PCR.⁸⁶ Protein expression was confirmed in skin from 4 hairdressers and 1 patient with ACD by using immunofluorescence staining.⁸⁶ Claudin-1 (*CLDN1*), *CLDN8*, *CLDN11*, CXADR-like membrane protein (*CLMP*), occludin (*OCLN*), membrane-associated guanylate kinase inverted 1 (*MAGI1*), and *MAGI2* mRNA expression was downregulated in patients with severe ACD. *CLDN1* and *CLMP* expression were downregulated in nonresponding hairdressers and patients with mild ACD. *FLG1*, *FLG2*, and *LOR* expression were downregulated in patients with ACD. Confocal microscopic images showed downregulation of CLDN-1, FLG-1, and FLG-2 expression. In contrast, 3-dimensional skin cultures showed upregulation of FLG-1 in

response to PPD but downregulation of FLG-2. This study has shown that PPD-exposed skin is associated with extensive transcriptomic changes, including downregulation of tight junction and stratum corneum proteins, even in the absence of clinical symptoms. Therefore, the net effect of hapten and antigen may vary depending on the severity of the disease in ACD. Further study is needed to validate the effect of PPD in intrinsic and extrinsic AD patients.

6. Role of new players in AD pathogenesis as modifiers of the cytokine microenvironment – the role of inflammatory fibroblasts and ILC2

Recent advances in experimental techniques have enabled the analysis of minor populations on a per-cell basis. Innate lymphoid cells (ILCs), minor lymphocytes, and fibroblasts have been suggested to play a role in AD development (Figure 3).

Innate immune cells are composed of granulocytes, macrophages, innate like T cells, and three groups of ILC (Table 2).^{87, 88, 90-98} In particular, ILC2 and basophils have been regarded as a source of type 2 cytokines in AD lesion.^{5, 53, 83, 87, 89-93} Additionally, cytokines classified as alarmins induce the expression of type 2 cytokines in AD lesions in an antigen-independent manner.⁵ Type 2 cytokines derived from activated ILC2 cause skin barrier disruption, which subsequently allows the entry of various antigens into the skin, leading to the induction of antigen-specific effector TH2 cells in the skin-draining lymph nodes.⁵ These effector TH2 cells present with accumulation in skin lesions and are activated by antigens or alarmins (such as IL-1, IL-18, and IL-33), thereby further amplifying inflammation.⁵ Therefore, the conventional perspective of AD pathology as a disease of acquired immunity presents with certain challenges.

ILC2 may not simply augment the TH2-cytokine axis. A per-cell basis study conducted on AD skin lesions revealed an unexpected complexity of the phenotype of ILC2.⁹⁹ ILC2 is present at a markedly lower frequency than T cells.⁹⁹ However, ILC2 has been found at increased levels in AD lesions compared with healthy control skin.⁸⁹ Keratinocytes in AD lesions produce IL-25 that activates ILC2.⁹⁹ Activated ILC2 produce IL-13 production

at sites of allergic skin inflammation in a murine model.⁹⁹ Analysis of skin punch biopsies obtained from AD or healthy individuals revealed that the majority of cutaneous ILC belonged to a CRTH2⁺ subset residing in the upper skin layers.¹⁰⁰ Single-cell RNA sequencing helped identify that cutaneous ILCs were predominantly ILC2.¹⁰⁰ However, such ILC2 seem protean because they co-express *GATA3*, *AHR*, and *RORC* in the inflammatory environment of AD skin lesions.¹⁰⁰ *GATA3* endows these lymphocytes with the capability to produce type 2 cytokines.¹⁰¹ *RORC* is the master transcription factor for ILC3, and TH17/TH22.¹⁰¹ *AHR* encodes an aryl hydrocarbon receptor. AHR recognizes xenobiotics, chemical compounds not naturally produced within the organisms (drugs, pesticides, and carcinogens).¹⁰²⁻¹⁰⁴ In response to such ligands, AHR functions as a critical transcription factor for the production of IL-22 in the ILC3 and TH17/TH22 subset.¹⁰¹ Such multivalent expression of transcription factors in a single ILC2 may explain the presence of activated TH17/TH22-cytokine axes in AD lesions.

Another possible role of ILC2 is in IL-10-mediated inhibition. In a retrospective, double-blind, placebo-controlled trial study, ILC2 has been found to produce IL-10 in patients who received grass-pollen sublingual immunotherapy.¹⁰⁵ Such inhibitory ILC2s express KLRG1 and produce IL-10 upon activation with IL-33 and retinoic acid.¹⁰⁵ Inhibitory ILC2 attenuates T cell responses and helps maintain nasal epithelial cell integrity *in vitro*.¹⁰⁵ However, whether this mechanism plays a role in AD is an aspect that warrants further study. Modification of the retinol metabolic pathway, cytokine-cytokine receptor interaction, and JAK-STAT signaling pathways in ILC2 may be deemed novel strategies for treating or preventing AD via allergen immunotherapy.

AHR is a key molecule in AD that helps modify the cytokine environment of the skin in response to haptens, foreign antigens, and cutaneous microbial metabolites. AHR is expressed by various cells such as epithelial cells, DCs, lymphocytes, ILC3, and CRTH2⁺ KLRG1⁺ ILC2 (described above).^{100, 106} The effect of AHR is situation-dependent.¹⁰⁶ AHR demonstrates the function of recognition of xenobiotic molecules. The typical xenobiotic ligands include 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).^{102, 103} TCDD plays immunosuppressive roles by inducing regulatory T cells (Tregs) and by inhibiting TH1, TH2, and TH17 differentiation.¹⁰³ Endogenous and physiological AHR

ligands are generated by cells, microbiota, or dietary sources, and exert effects on lymphocyte development and function.¹⁰⁴ The high-affinity AHR ligand 6-formylindolo[3,2-b]carbazole (FICZ) is an ultraviolet photoproduct of L-tryptophan.¹⁰⁴ FICZ is also generated via other metabolic pathways, including enzymatic deamination of tryptamine and oxidation of tryptophan by intracellular oxidants.¹⁰⁷ FICZ promotes TH17 differentiation and increases the numbers of gut ILC3 in an AHR-dependent way.¹⁰⁴ Indolo-[3,2-b]-carbazole (ICZ) is another high-affinity ligand generated with 3,3'-diindolylmethane from indole-3-carbinal (I3C) under acidic conditions in the stomach.¹⁰⁴ I3C is enzymatically generated from L-tryptophan-derived glucosinolate present in cruciferous vegetables.¹⁰⁴ An AHR agonist, kynurenine, is a tryptophan metabolite generated by the activity of the enzymes indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO).¹⁰⁴ IDO is induced by AHR.¹⁰⁴ The effect of activated AHR differs depending on the environment as represented by FICZ and kynurenine.¹⁰⁸ Kynurenine promotes TGF β -induced Treg differentiation, whereas FICZ demonstrates an inhibitory function.¹⁰⁹ FICZ and TCDD affect lineage specification of TH17 and Treg via different mechanisms.¹⁰⁸ Additionally, microbe-derived molecules function to activate AHR. The cutaneous commensal yeast *Malassezia* metabolizes tryptophan to several AHR ligands, including FICZ and ICZ.¹¹⁰ Therefore, AHR plays a multifaceted role in dermatitis. Understanding the role of AHR may lead to the formulation of a novel treatment regimen for AD.

NK cells play roles in AD. Although NK cells belong to ILC1, several differences exist between NK cells and other ILC1 cells.^{87, 88} NK cells are cytotoxic, whereas other ILC1 cells are not.⁸⁸ The development and maintenance of NK cells and other ILC1 depend on IL-15 and IL-7, respectively.^{87, 88} The role of NK cells in AD has been reported.^{111, 112} The lesions of chronic AD, Asian AD, and intrinsic AD contain TH1 and TH2, which further aid the production of IFN- γ . Although the exact role of such TH2-counteracting cytokines remains unknown, a study has proposed the presence of an endogenous mechanism that helps maintain cutaneous immune homeostasis via functions of NK cells.^{111, 112} NK cells promote antiviral immunity through cytotoxic activity and the production of IFN- γ . The authors observed decreased levels of peripheral blood NK cells and altered composition of NK cell subsets, characterized by a reduction of mature

CD56^{dim} CD57⁺ NK cells.¹¹¹ This subset of NK cells produced substantial amounts of IFN- γ in a high proportion of an adult patient cohort with moderate-to-severe AD.¹¹¹ Activated NK cells were found to be enriched in lesional AD skin biopsies.¹¹¹ These data implied that the features of low blood NK cell numbers could be considered to be of a certain diagnostic value and could be used to effectively distinguish AD from chronic pruritus of unknown origin.¹¹²

Natural killer T (NKT) cells are recognized as unconventional T cells that bridge innate and adaptive immunity. Histologically, AD is characterized by dermal infiltration of adaptive immune cells and innate immune cells (eosinophils, mast cells, and invariant NKT cells).¹¹³ NKT cells promote survival and maturation of DCs in the draining LNs in the sensitization phase of the CHS model in mice.¹¹⁴ The CHS response is attenuated in *Cd1d1*^(-/-) and *Traj18*^(-/-) BALB/c mice in which NKT cells are absent.^{111, 112} In patients with AD, expression levels of CXCR4 and its ligand CXCL12 were upregulated in the skin as evidenced by global transcriptomic and proteomic analyses.¹¹⁵ Accordingly, CXCR4⁺ NKT cells were observed to be enriched in AD skin.¹¹⁵ Skin fibroblasts were the main source of CXCL12.¹¹⁵ This line of evidence suggested NKT as another modifier of the pathogenesis of AD.

Fibroblasts or fibroblast-like cells play a role in several inflammatory diseases.¹¹⁶⁻¹¹⁸ Single-cell RNA sequencing of skin biopsy specimens derived from five patients with AD (four lesional samples and five nonlesional samples) and seven healthy control subjects was performed, and unique populations of fibroblasts were identified.¹¹⁹ A *COL6A5*⁺*COL18A1*⁺ subpopulation was found to be unique to lesional AD and expressed *CCL2* and *CCL19*. A corresponding LAMP3⁺ DC population expressing the *CCL19* receptor CCR7 was also observed to be unique to AD lesions. These findings illustrated a potential role for fibroblast signaling in immune cells. The lesional AD samples were characterized by the expansion of inflammatory DCs (*CD1A*⁺ and *FCER1A*⁺) and tissue-resident memory T cells (*CD69*⁺ and *CD103*⁺). A unique inflammatory fibroblast may establish interactions with immune cells to regulate lymphoid cell organization and type 2 inflammation.¹¹⁹

7. Comorbidities and endotypes of AD – association with cardiovascular diseases

In addition to immune cells, comorbidities and metabolic states may affect the formation of endotypes of AD.¹²⁰⁻¹²² Increasing AD severity is associated with increased risk of cardiovascular outcomes.¹²⁰ Obesity may affect the severity of AD.¹²³ A meta-analysis involving 33 published studies on the association between obesity and AD showed an odds ratio of 1.19 (95% confidence interval [CI] = 0.95–1.49) in adults (n = 1,400,679) and 1.20 (95% CI = 1.11–1.30) in children (n = 796,514).¹²³ These findings indicate that the adipocyte-keratinocyte cross-talk or metabolic status may be considered in the development of a future AD treatment strategy.¹²³

The prevalence of metabolic syndrome and its components was evaluated in another large group of patients with AD compared to a matched reference group.¹²⁴ This was a cross-sectional study involving AD patients diagnosed by a dermatologist between 1998 and 2016, and a matched comparison group.¹²⁴ The association between AD and metabolic syndrome, its components, and possible complications for the entire study population, including 116,812 comparison adults (age > 18) and 116,816 adults with moderate-to-severe AD, were analyzed.¹²⁴ AD in the entire group of patients and the adult patients was associated with a higher prevalence of dyslipidemia and a lower prevalence of diabetes and metabolic syndrome.¹²⁴ Moderate and severe AD cases were associated with higher prevalence rates of metabolic syndrome (17.0% vs. 9.4%), its components (obesity: 22.2% vs. 18.6%; diabetes: 15.9% vs. 9.2%; hypertension: 27.9% vs. 15.3%; dyslipidemia: 47.1% vs. 28.5%) and cardiovascular morbidity.¹²⁴

However, another systematic review indicates inconsistency, especially concerning the association between AD and hypertension; a positive association between AD and hypertension was reported in six out of the eleven included studies.^{125, 126} An inverse association was found in three studies, and no association was noted in two studies.^{125,}

¹²⁶ A potential confounding factor that might be partially responsible for such inconsistency was related to the criteria used for defining metabolic syndrome and

hypertension in the studies.¹²⁶ Further evaluation will be necessary to understand the association between AD and metabolic syndrome comprehensively.

8. Aging and AD – evolving T cell subsets with aging

Aging is another factor that result in modification of the molecular phenotype of AD (Figure 4).¹²⁷ Rates of self-reported AD remain high after childhood, and adult-onset AD has different risk factor associations.⁷³ Pathologic T cell subsets also evolve with aging.⁶⁹ A comparison using flow cytometry was performed for elucidating immune activation and cytokine polarization in the blood of 0-5-year-old (n = 39), 6-11-year-old (n = 26), 12-17-year-old (n = 21), and 18-year-old or older (n = 43) patients with AD versus age-matched control subjects.⁶⁹ CLA⁺ TH1 frequencies were lower in infants with AD than those in all older patients (P < .01).⁶⁹ The frequencies of CLA⁺ TH2 T cells increased across all AD age groups compared with the control subjects (P < 0.05).⁶⁹ After infancy, CLA⁺ TH2 frequencies were increased in patients with AD in all age groups, suggesting systemic immune activation with disease chronicity.⁶⁹ IL-22 frequencies serially increased from normal levels in infants to highly significant levels in adolescents and adults compared with the respective control subjects (P < 0.01).⁶⁹ The adult AD phenotype is achieved only in adulthood. Unique cytokine signatures characterizing individual pediatric endotypes may be countered via age-specific therapies.

Cytokine profiles continue to undergo alterations in mature adults in an age-specific manner (Figure 5). A study evaluated age-specific changes in lesional and nonlesional tissues and blood derived from patients with moderate-to-severe AD (n = 246) and age-matched control subjects (n = 71) (Figure 5).¹²⁷ Disease severity/SCORAD scores were similar across AD age groups.¹²⁷ Infiltration of CD1b⁺ or FcεRI⁺ DCs decreased with age.¹²⁷ TH2 levels (*IL5*, *IL13*, *CCL13*, *CCL18*, and *CCL26*) decreased with age in patients with AD, despite increasing in the control group.¹²⁷ Serum IgE levels and eosinophil counts were negatively correlated with age in patients with AD.¹²⁷ *IL22* expression levels also decreased with age in AD patients.¹²⁷ Expression of TH1-related

(*IFNG*, *IL12/23p40*, *STAT1*, and *CXCL9*) and TH17-related (*IL17A* and *IL20*) markers increased with age in both AD patients and control subjects.¹²⁷ The expression of keratinocyte terminal differentiation markers increased in older AD patients (LOR and FLG).¹²⁷ In contrast, the expression of S100A8 and hyperplasia markers (epidermal thickness, keratin 16, and Ki67) decreased.¹²⁷ Serum trends in AD mimicked trends observed in skin, with the exhibition of TH2 downregulation (CCL26; $r = -0.32$, $P < 0.1$) and TH1 upregulation (IFN γ ; $r = 0.48$, $P < 0.01$) with age.¹²⁷ Therefore, the adult AD profile varied with age. Although TH1/TH17 skewing increases in both patients with AD and control subjects, patients with AD demonstrate unique decreases in TH2/TH22 polarization and normalization of epithelial abnormalities. Thus, age-specific treatment approaches may be beneficial for AD.

9. The microbiome and AD – a link between TSLP and microbe-derived tryptophan metabolites

The skin microbiota can contribute to AD pathogenesis.^{85, 128-137} In one study, 30% of the patients with AD experienced bacterial or viral infection, whereas this complication occurred in only 7% of the patients with psoriasis.¹³⁸ Skin lesions in >90% of the patients with AD were reportedly colonized with *Staphylococcus aureus*, and the density of *S. aureus* was associated with AD severity in other studies.^{139, 140} DNA/RNA-sequencing technologies have revealed that the skin microbiota changes as dermatitis worsens.¹⁴¹ Therefore, alteration of the cutaneous microbiota (dysbiosis) can affect the course of AD in a manner analogous with gut microbiota alteration-associated exacerbations reported in inflammatory bowel diseases.¹⁴¹ However, the mechanism by which dysbiosis affects the integrity of the skin resulting in dermatitis remains unclear.

Such a phenomenon of dysbiosis-mediated dermatitis may be explained by considering a tryptophan metabolite of the skin microbiota.¹⁴² A study was conducted on tryptophan metabolites on the skin surfaces of patients with AD.¹⁴² Skin surface metabolites were collected using the gel-patch method.¹⁴² Samples were assessed using liquid chromatography-tandem mass spectrometry (LC-MS/MS).¹⁴² Major microbial

metabolites of tryptophan were detected on the skin surfaces of healthy subjects.¹⁴² The level of indole-3-aldehyde (IAld), an indole derivative of tryptophan catabolism, was lower in the lesional and nonlesional skin of patients with AD than that reported in healthy subjects.¹⁴² IAld significantly attenuated skin inflammation in mice with MC903-induced AD-like dermatitis, and this effect was blocked by an AHR antagonist and abolished in AHR-null mice.¹⁴² Furthermore, IAld was found to inhibit the MC903-induced expression of TSLP in keratinocytes *in vivo* and *in vitro*, and the mechanism was mediated by the binding of AHR to the TSLP promoter.¹⁴² The mechanism how AHR-expressing immune cells (ILC and TH17/TH22) are involved awaits further study.

Another factor in AD development is the toxins produced by the microbes on the skin. *S. aureus* second immunoglobulin-binding protein (Sbi) has been proposed to drive AD via IL-33.¹⁴³ An *in vitro* human keratinocyte cell culture, *ex vivo* human skin organ explants, and the eczema-prone Nishiki-nezumi Cinnamon strain mouse were used as model systems. A mutant strain with Sbi confirmed its ability to induce the rapid release of constitutive IL-33 from human keratinocytes, independent of the Toll-like receptor pathway.¹⁴³ Its ability could be related to the disruption of corneodesmosin, a component of corneodesmosomes that form key intercellular adhesive structures in the stratum corneum.¹⁴³ These lines of evidence indicate that dysbiosis of skin microbes also affects the phenotype of AD.

10. Conclusions

AD presents with a substantial socioeconomic burden in every country. Recent pathogenic discoveries have revealed that AD is composed of unexpectedly complex endotypes. Although TH2 axis activation characterizes the inflammation of AD, other cytokine axes are also activated and may possibly modify the course of the disease. The relative contributions of each cytokine axis in driving different endophenotypes are being investigated. Future clinical trials may help stratify patients based on their endotypes before treatment. Such efforts will result in the establishment of better optimized therapeutic protocols for each patient with AD.

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1092 Table 1. Barrier and cytokine changes reported in atopic dermatitis by race.

Features		European	Asian	African
Barrier	Thickness	↑↑	↑↑↑	↑↑
	K16	↑	↑↑	↑
	Ki67	↑	↑↑	↑
	FLG	↓↓↓	↓	→
	LOR	↓↓↓	→†	↓↓
Cytokine	TH2	↑↑↑	↑↑↑	↑↑↑
	TH22	↑↑↑	↑↑↑↑	↑↑
	TH17	↑	↑↑↑	Absent
	TH1	↑↑	↑~↑↑	Absent

1093 Simplified characteristics of atopic dermatitis in each race are shown.

1094 Upward arrow (↑), horizontal arrow (→), and down arrow (↓) indicate upregulation, no
 1095 difference, and downregulation in skin lesions in comparison with healthy controls. The
 1096 number of arrows indicates the extent of this degree. † LOR was decreased in Han
 1097 Chinese in one study.⁵⁵ Abbreviations: K16, keratin 16; FLG, filaggrin; LOR, loricrin.

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1100 Table 2. Simplified view of innate lymphoid cells and innate like T cells.

	Innate lymphoid cells (ILC)					Innate-like T cells		
	NK	ILC1	ILC2	ILC3	LTi	$\gamma\delta$ T	NKT	MAIT
TCR	-	-	-	-	-	$\gamma\delta$	$\alpha\beta$	$\alpha\beta$
Cytokine production	IFN- γ , TNF		IL-4, IL-5, IL-13	IL-17, IL-22		IFN- γ , TNF, IL-17	IFN- γ , TNF, IL-17, IL-22	IFN- γ , TNF, IL-17, IL-22
Main TFs	TBX21, EOMES	TBX21?	GATA3, RORA	RORC, AHR	RORC	PLZF	PLZF	PLZF, RORC, EOMES
Analogues	TC1	TH1	TH2	TH17, TH22	†	TH1, TH17	TH1, TH2, TH17, TH22	TH1, TH17, TH22

1101 Current view of ILC and innate-like T cells.

1102 Abbreviations: NK, natural killer; LTi, lymphoid tissue inducer; MAIT, mucosa associated
 1103 invariant T.

1104 †; Whether the cytokines produced by LTi play roles in adaptive immunity is not clear.

1105

1106 Figure legends

1107 **Figure 1. Four clusters identified by 143 biomarkers.**

1108 Serum samples obtained from 146 patients with severe AD (median EASI = 28) were
1109 analyzed for a panel of 143 biomarkers.⁷⁶ Four clusters have been identified (see main
1110 text for detail).

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1112

1113

Figure 2. Differential dermatitis outcomes by different skin barrier conditions.

Small molecules, such as metal particles and hapten, may lead to intrinsic AD development. Hapten exposure provokes TH1 initially, followed by TH2 production on repeated exposure. Alternatively, the shift from TH1 to TH2 may be induced by basophils, immune cells which express MHC class II and IL-4 in the draining lymph nodes. Protein antigens are captured by Langerhans cells in the epidermis that induce TH2 via TSLP-TSLP receptor (TSLPR) signaling.

1125 **Figure 3. New players of atopic dermatitis pathogenesis.**

1126 In addition to TH2/TC2, various types of cells play roles in AD skin lesion development
1127 (see main text for details).^{100, 111, 112, 114, 115, 119}

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Figure 4. Aging and the cytokine profile of peripheral blood.

The peripheral blood immune phenotype in children and adults with or without atopic dermatitis is shown. Czarnowicki et al. reported the use of flow cytometry to measure cytokine levels associated with TH1 (IFN- γ), TH2 (IL-13 and IL-9), TH17 (IL-17), and TH22 (IL-22) in CD4⁺ and CD8⁺ T cells, and the number of regulatory T cells (Tregs).⁶⁹

1139 **Figure 5. Age-specific cytokine profiles in mature adults.**

1140 The peripheral blood immune phenotype in children and adults with or without atopic
1141 dermatitis is shown.¹²⁷

1142

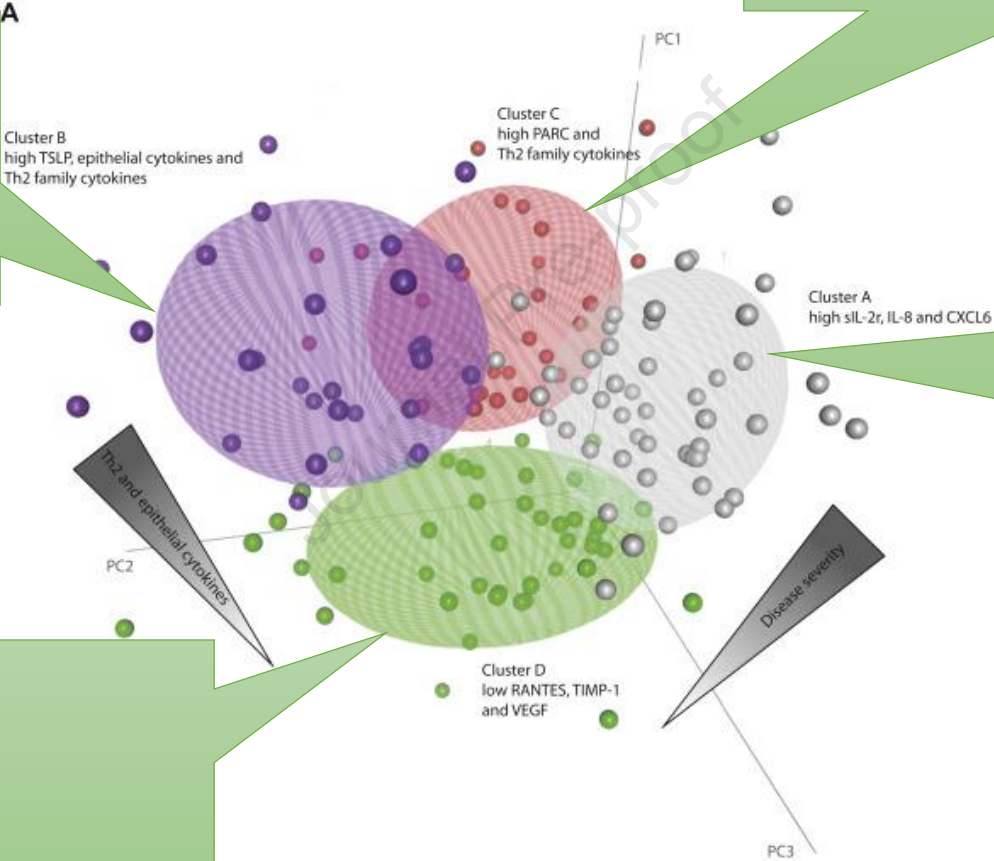
Four clusters of AD

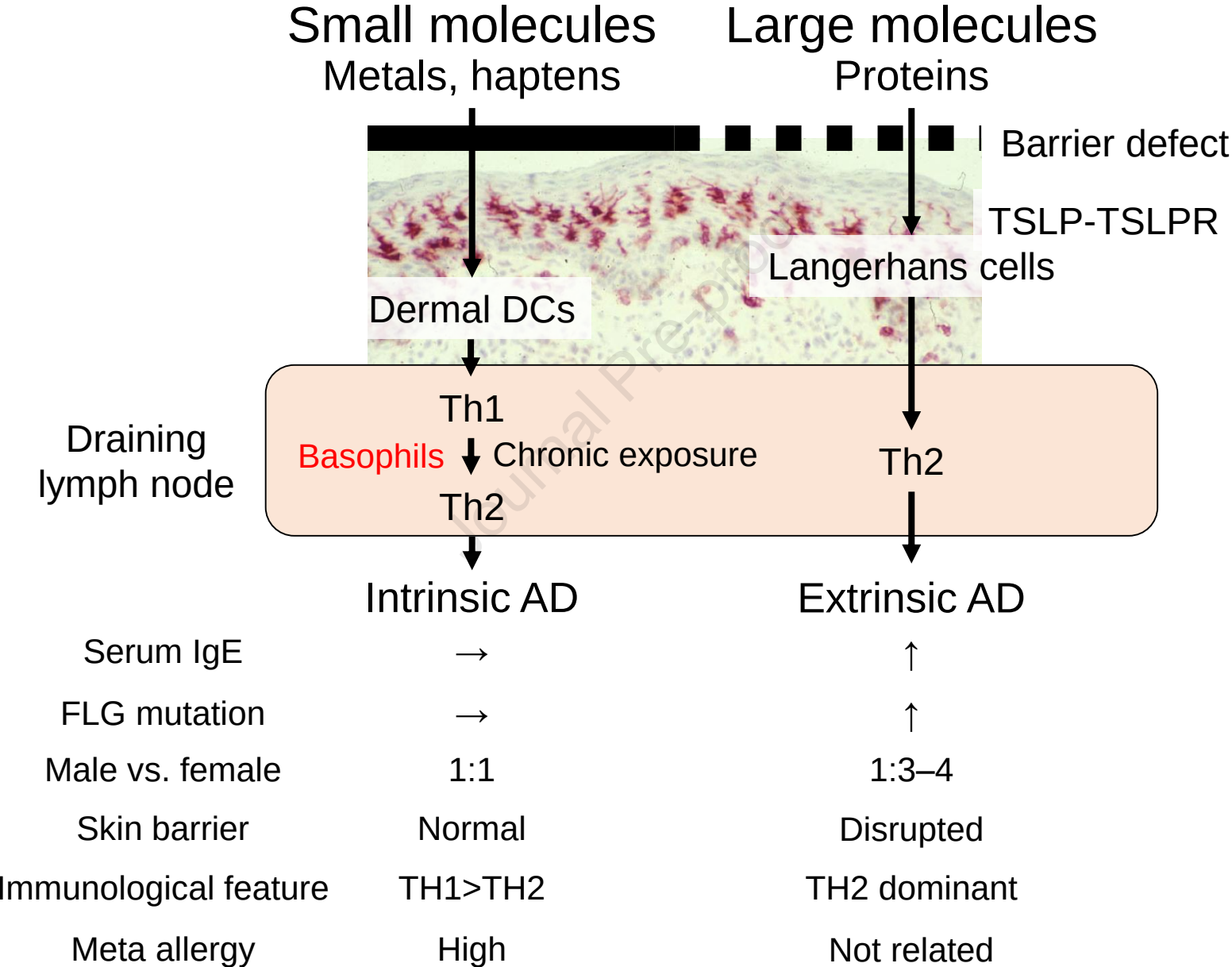
B (18%, EASI 25)
•TH1/TH2/TH17-dominant
•Often seen in Asian AD

C (18%, EASI 38)
•TH2/TH22/PARC-dominant
•Severe AD

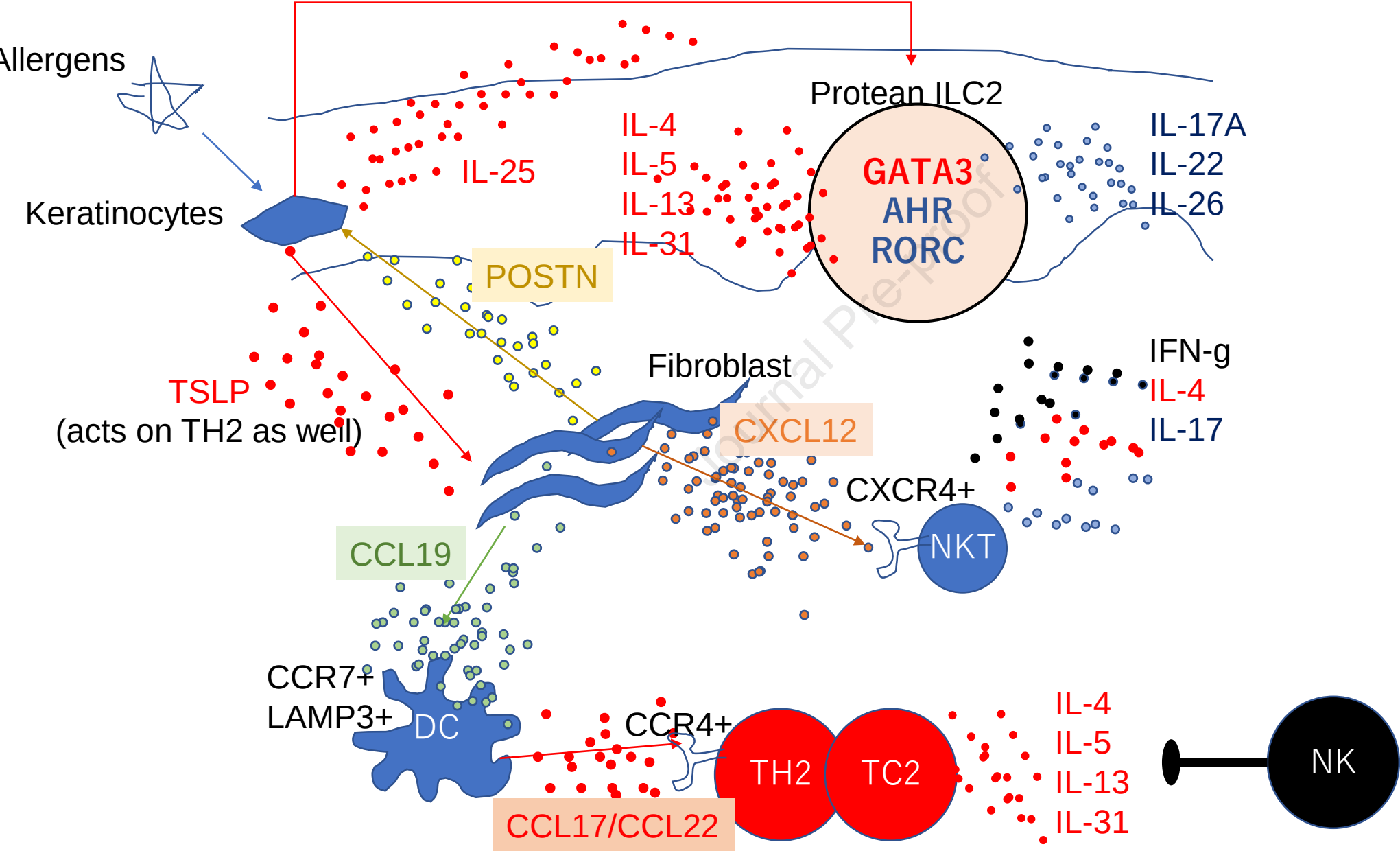
A (34%, EASI 28)
•TARC/IL-1R1-dominant
•Previous immunosuppressants

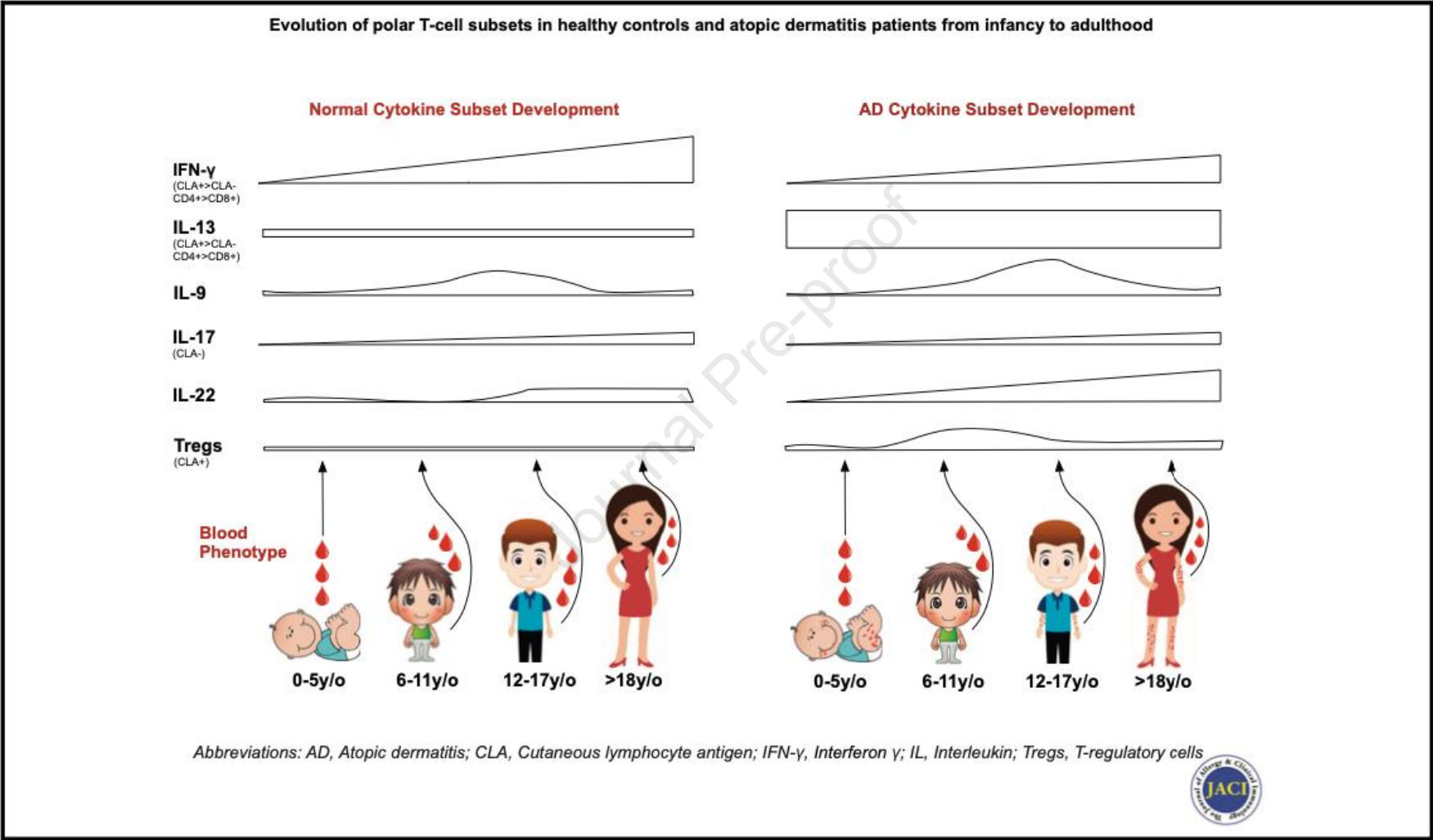
D (30%, EASI 27)
•TH2/eosinophil-inferior
•Intrinsic AD?





Nomura and Kabashima
Figure 3





Adapted from:
Evolution of pathologic T-cell subsets in patients with atopic dermatitis from infancy to adulthood
T. Czarnewicki, H. He, T. Canter, J. Han, R. Lefferdink, T. Erickson, et al.
J Allergy Clin Immunol 2020 Vol. 145 Issue 1 Pages 215-228

Figure 5

