Atopic dermatitis biomarker analysis points to elevation of TSLP and IL-33 signaling and suggests a role for type 2 innate lymphoid cells

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Introduction

- Atopic dermatitis (AD) is a chronic, inflammatory skin disease characterized by a disrupted epithelial barrier that predisposes skin to penetration by microbes and epicutaneous allergens[1–3]
  - Widespread, relapsing skin lesions occur in AD, yet nonlesional skin may also show signs of (less severe) inflammation[4]
- Epithelial-derived "danger signaling" cytokines called alarmins are released in damaged skin[5,6]
  - Alarmins act via receptors on dendritic cells, type 2 innate lymphoid cells (ILC2s), and mast cells (among other cell types) to induce a potent T helper type 2 (T2)–driven response in a localized or systemic manner[5–9]
- Profiling alarmin-related genes and signatures in key physiological compartments may provide robust measures of alarmin activity and mechanistic insight into AD
- Thymic stromal lymphopoietin (TSLP) and interleukin (IL)-33 are 2 alarmin cytokines expressed near barrier surfaces, including skin and bronchial epithelium[6,8,9]
  - AD therapies that target IL-33 and TSLP pathways are in clinical development

[a]A poster reporting a highly sensitive assay to measure blood TSLP is presented at this congress (#091).

Objective

In patients with AD, we adopted a cross-tissue approach to assess alarmin biology in skin, sampled by biopsies and noninvasive skin tape strips that measure epidermal immunopathology, and in blood

Methods

Study populations and sample collection

- Skin punch biopsies, epidermal skin tape strip samples, and whole blood were collected from patients with physician-diagnosed AD and healthy individuals without atopic disease
  - In patients with AD, biopsies and tape strip samples were collected from lesional and nonlesional sites 2 weeks after stopping topical corticosteroids
- AD severity status was determined by Rajka Langeland and Eczema Area and Severity Index (EASI) scores[a]

Profiling genes, proteins, and immune cell signatures

- RNA was extracted from skin biopsy, whole blood, and skin tape strip samples and analyzed by RNA-seq (biopsy and blood) or qPCR (skin tape strips)
- Gene signatures of cells commonly associated with AD were measured,[b] including:
  - An alarmin-induced ILC2 signature (ILC2 cells were expanded from PBMCs and stimulated with IL-33/TSLP+IL-33 for 6 hours)[c]
  - A TSLP-induced dendritic cell signature (adapted from Ito et al)[10]
  - A non-induced mast cell signature (previously reported)[11]
- Genes/signatures were considered significantly altered if they were at least 1.5-fold different between groups, with a statistically significant *P*-value (ANOVA with post hoc comparisons between 3 groups; paired *t*-test[a] for lesional vs nonlesional samples)
- Blood protein levels were assessed using the SOMAscan multiplex proteomic method. Statistical significance was determined by *t*-test
- Protein extracts from skin tape strips[a] were analyzed by targeted multiplexed quantitation using liquid chromatography-mass spectrometry (LC-MS).[12] Statistical significance was determined by ANOVA (3 groups) and Mann-Whitney U test (2 groups)

PBMC, peripheral blood mononuclear cell; qPCR, quantitative polymerase chain reaction. [a]Mild to moderate AD, EASI score <21.0; severe to very severe AD, EASI score ≥21.0. [b]Gene signatures were obtained under in vitro conditions that did not specifically use skin cells. [c]Expanded under IL-2+IL-1β for 10 days. [d]Paired *t*-test with false discovery rate determined for multiple comparisons; [e]DermTech skin tape strips were used for qPCR, and CuDerm skin tape strips were used for peptide analysis.

Results

Demographics and clinical characteristics

- Demographics were similar between patients with AD and healthy individuals (**Table 1**)
- Total and specific serum immunoglobulin E levels were significantly higher in patients with AD compared with healthy individuals (*P*<0.05); however, blood eosinophils were not significantly different (**Table 1**)

Table 1. Demographics and clinical characteristics of patients with AD and healthy individuals without atopic disease

Characteristic	Patients with AD (n=43)	Healthy individuals without atopic disease (n=10)
Age, years[a]	35 [20–63]	41 [26–59]
Men, n (%)	22 (51)	4 (40)
Ethnicity, n (%) White African American Other	 24 (56) 7 (16) 12 (28)	 6 (60) 1 (10) 3 (30)
Mild-to-moderate AD, n (%)	26 (60)	–
Severe-to-very-severe AD, n (%)	17 (40)	–
Rajka Langeland eczema severity score[a]	8 [6–9]	–
EASI score[a]	16.8 [5.8–69.0]	–
Blood eosinophils, %[a]	2.9 [1.0–8.6]	1.2 [0.5–3.2]
Blood eosinophils, cells/μL[a]	200 [70–680]	100 [30–950]
Total IgE, kIU/L[a]	162 [2–20,976]	10 [2–67]
ImmunoCAP IgE, kUA/L[a]	9.5 [0.1–906.0]	0.1 [0.1–4.2]
Staphylococcus aureus status[b] positive/negative, n Lesional skin Nonlesional skin	 20/23 14/29	 – –

AD, atopic dermatitis; EASI, Eczema Area and Severity Index; Ig, immunoglobulin; MRSA, methicillin-resistant *Staphylococcus aureus*.
[a]Median [range]. [b]*Staphylococcus aureus* was not detected in any healthy individuals. MRSA was detected using skin swabs in 3 lesional skin samples and in no nonlesional skin samples from patients with AD.

Genes upregulated in lesional vs nonlesional skin biopsy samples from patients with AD

- *CCL17*, *CCL18*, and *CCL22* T2-attracting chemokines[a] were among the top 25 significantly upregulated genes in lesional skin (n=35) compared with nonlesional skin (n=35) in biopsies from patients with AD (**Table 2**)

[a]*CCL17* and *CCL22* expression is closely associated with dendritic cell response to TSLP activation.[13]

Table 2. Top genes upregulated in AD lesional vs nonlesional skin biopsies

Gene	AD lesional skin vs AD nonlesional skin (n=35)[a]	
	Fold difference	False discovery rate
CCL17	2.78	0.05
CCL18	2.62	0.04
CCL22	2.35	0.04
Other upregulated genes (in descending order of fold difference; range, 9.74–2.26): *SERPINB4*, *KRT6C*, *S100A8*, *S100A9*, *SPRR2A*, *KRT16*, *PI3*, *KRT6A*, *SERPINB3*, *S100A7*, *SPRR1A*, *IGFL1*, *MMP12*, *OASL*, *PRSS22*, *LTF*, *UGT1A7*, *SPRR2B*, *AKR1B10*, *PLA2G2A*, *SPRR1B*, *RGS1*.[b]		

AD, atopic dermatitis.
[a]Eight of 43 patients were excluded because matched lesional and nonlesional skin biopsy samples were not available; [b]False discovery rate, <0.05.

Upregulated expression of genes/proteins in skin biopsy and blood samples from patients with AD

- *CCL17*, *CCL18*, and *MMP12* were upregulated in skin biopsies (specifically in lesional skin) (n=35) and also in blood (n=43) from patients with AD compared with healthy individuals (n=10) (*P*<0.05) (**Table 3**)

Table 3. Upregulated expression of genes/proteins in skin biopsy and blood samples from patients with AD vs healthy individuals

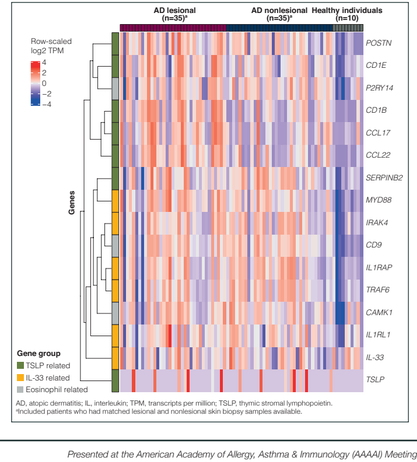
Gene/ protein	Patients with AD vs healthy individuals[a]	
	Skin biopsy (lesional skin, n=35)[b] Gene expression fold difference	Blood (n=43) Protein expression fold difference
CCL18	25.81	1.54
MMP12	7.23	1.57
CCL17	5.18	1.56

AD, atopic dermatitis.
[a]N values are for patients with AD; healthy individuals (n=10); [b]Included patients who had matched lesional and nonlesional skin biopsy samples available.

Alarmin-related gene expression in skin biopsies from patients with AD

- Genes known to be induced downstream of alarmins were upregulated in patients with AD (n=35) vs healthy individuals (n=10) (**Figure 1**)
  - Some TSLP-related genes (including *CCL17*, *CCL22*, *CD1B*) had higher expression levels in lesional vs nonlesional skin
  - IL-33– and eosinophil-inducible genes were upregulated to a similar extent in all AD skin, inclusive of lesional and nonlesional skin

Figure 1. Heat map analysis of gene expression in AD skin biopsies[a]

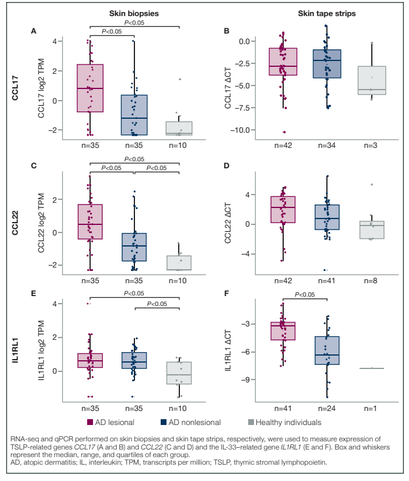


AD, atopic dermatitis; IL, interleukin; TPM, transcripts per million; TSLP, thymic stromal lymphopoietin.
[a]Included patients who had matched lesional and nonlesional skin biopsy samples available.

Elevation of TSLP- and IL-33–related genes in skin biopsies and skin tape strips from patients with AD

- In skin biopsies, transcript levels of CCL17 and CCL22 were elevated in patients with AD compared with healthy individuals (*P*<0.05) (**Figure 2A–D**)
  - A similar nonsignificant trend was observed in skin tape strip samples
- In skin biopsies, transcript levels of IL1RL1 (ST2, a subunit of the IL-33 receptor) were elevated in patients with AD compared with healthy individuals (*P*≤0.05)
  - Expression of IL1RL1 transcripts in lesional or nonlesional skin was similar (**Figure 2E**); however, in skin tape strips, transcripts showed preferential elevation in lesional vs nonlesional skin (*P*<0.05) (**Figure 2F**)

Figure 2. TSLP- and IL-33–related genes in skin biopsies and in skin tape strips

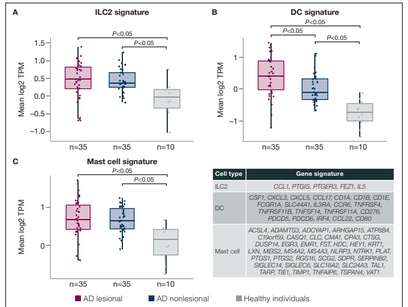


RNA-seq and qPCR performed on skin biopsies and skin tape strips, respectively, were used to measure expression of TSLP-related genes *CCL17* (A and B) and *CCL22* (C and D) and the IL-33–related gene *IL1RL1* (E and F). Box and whiskers represent the median, range, and quartiles of each group.
AD, atopic dermatitis; IL, interleukin; TPM, transcripts per million; TSLP, thymic stromal lymphopoietin.

Alarmin-induced ILC2 and dendritic cell gene signatures and non-induced mast cell gene signatures in skin biopsy samples from patients with AD

- ILC2s, dendritic cells, and mast cells express alarmin receptors.[4–6] Elevated immune cell gene signatures were observed in skin biopsy samples from patients with AD (n=35) compared with healthy individuals (n=10), including signatures derived from:
  - TSLP+IL-33–stimulated ILC2s (*P*<0.05) (**Figure 3A**); TSLP-stimulated dendritic cells (*P*<0.05) (**Figure 3B**); and non-induced mast cells (*P*<0.05) (**Figure 3C**)

Figure 3. Gene signature profiles of alarmin-induced ILC2s and dendritic cells and non-induced mast cells



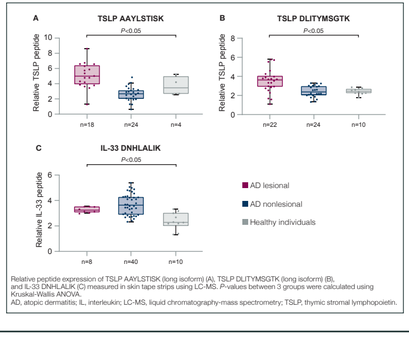
Cell type	Gene signature
ILC2	*CCL1, PTGIS, PTGER3, REC1, IL5*
DC	*CSF1, CXCL5, CXCL6, CCL17, CD1A, CD1B, CD1E, FCGR1A, SLC44A1, IL3RA, CCR6, TNFRSF4, TNFRSF11B, TNFSF14, TNFRSF11A, CD276, FOXQ1, FOXD4L, IRF4, CCL22, CD80*
Mast cell	*ACSL4, ADAMTS3, ADCYAP1, AIF1L, AIPL1, ATP1B4, C19orf59, CASQ1, CLC, CMA1, CPA3, CTSG, DUSP14, EGR3, EMR1, FST, HDC, HEY1, KRT1, LXN, MEIS2, MS4A2, MS4A3, NLRP3, NTRK1, PLAT, PTGS1, PTGS2, RGS16, SCG2, SDPR, SERPINB2, SIGLEC14, SIGLEC6, SLC18A2, SLC24A3, TAL1, TARP, TIE1, TIMP1, TNFAIP6, TSPAN4, VAT1*

Immune cell gene signatures derived from TSLP+IL-33–induced ILC2s (A), TSLP-induced dendritic cells (B), and non-induced mast cells (C) were measured in skin biopsies using RNA-seq. Box and whiskers represent the median, range, and quartiles of each group.
AD, atopic dermatitis; DC, dendritic cell; IL, interleukin; ILC2, type 2 innate lymphoid cells; TPM, transcripts per million; TSLP, thymic stromal lymphopoietin.

Increased TSLP and IL-33 peptide expression measured in skin tape strip samples from patients with AD

- TSLP peptide expression was elevated in patients with AD (specifically DLITYMSGTK in lesional skin) compared with healthy individuals (*P*<0.05; Mann-Whitney U test) (**Figure 4A–B**)
- IL-33 peptide levels were elevated in patients with AD (in lesional and nonlesional skin) compared with healthy individuals (*P*<0.05; Mann-Whitney U test) (**Figure 4C**)

Figure 4. Increased TSLP and IL-33 peptide expression measured in skin tape strip samples from patients with AD



Relative peptide expression of TSLP AAYLSTISK (long isoform) (A), TSLP DLITYMSGTK (long isoform) (B), and IL-33 DNHLALIK (C) measured in skin tape strips using LC-MS. *P*-values between 3 groups were calculated using Kruskal-Wallis ANOVA.
AD, atopic dermatitis; IL, interleukin; LC-MS, liquid chromatography–mass spectrometry; TSLP, thymic stromal lymphopoietin.

Conclusions

- Comprehensive assessment of gene expression has identified alarmin-related genes that serve as indicators of the cellular drivers of dysregulated skin and systemic inflammation in AD
  - IL-33–related genes were upregulated to a similar extent in nonlesional skin compared with lesional skin, consistent with systemic perturbations in the T2 axis in AD
  - Some TSLP-related genes had higher expression in lesional skin compared with nonlesional skin, suggesting localized dysregulation in AD lesions
- TSLP and IL-33 protein levels in the epidermis, as measured by LC-MS of skin tape strip extracts, were increased in patients with AD compared with healthy individuals
- Further evidence for upregulated alarmin-signaling pathways in AD is demonstrated by elevated gene signatures of alarmin-responsive cells (mast cells, dendritic cells, and ILC2 cells) in patients with AD compared with healthy individuals, although a limitation of the study is that gene signatures were generated in vitro
- Results support the rationale to therapeutically target alarmins or their respective receptors or signaling pathways to treat patients with AD. Alarmin-associated gene signatures may offer the potential to stratify patients with AD according to their immunopathology and identify patients who may respond to anti-alarmin therapies

References

1. Margolis JS, et al. *JAMA Dermatol*. 2014;150:593–600.
2. Bin L, Leung DY. *Allergy Asthma Clin Immunol*. 2016;12:52.
3. Leung DY, Guttman-Yassky E. *J Allergy Clin Immunol*. 2014;134:769–779.
4. Brunner PM, et al. *Allergy*. 2017;72:2017–2025.
5. Klonowska J, et al. *Int J Mol Sci*. 2018;19.
6. Yang D, et al. *Immunol Rev*. 2017;280:41–56.
7. Schmitz J, et al. *Immunity*. 2005;23:479–490.
8. Leyva-Castillo JM, et al. *Nat Commun*. 2013;4:2847.
9. Wang W, et al. *J Immunol*. 2018;201:2221–2231.
10. Ito T, et al. *J Exp Med*. 2005;202:1213–1223.
11. Charoentong P, et al. *Cell Rep*. 2017;18:248–262.
12. Erickson BK, et al. *Mol Cell*. 2017;65:361–370.
13. Soumelis V, et al. *Nat Immunol*. 2002;3:673–680.

Disclosures

JMG, **SS**, **MG**, **THP**, **YO**, **MR**, **FP**, and **IK** are employees of AstraZeneca. **JRP** and **SK** are employees of Amgen Inc. **CB** is an employee of National Jewish Health. **DYML** and **EG** are employees of National Jewish Health and were recipients of a grant from AstraZeneca to support a portion of this research. **DYML** has consulted for Janssen Pharmaceuticals, Regeneron, and Sanofi.

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