

Intrinsic atopic dermatitis shows similar T_H2 and higher T_H17 immune activation compared with extrinsic atopic dermatitis

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Background: Atopic dermatitis (AD) is classified as extrinsic and intrinsic, representing approximately 80% and 20% of patients with the disease, respectively. Although sharing a similar clinical phenotype, only extrinsic AD is characterized by high serum IgE levels. Because most patients with AD exhibit high IgE levels, an “allergic”/IgE-mediated disease pathogenesis was hypothesized. However, current models associate AD with T-cell activation, particularly T_H2/T_H22 polarization, and epidermal barrier defects.

Objective: We sought to define whether both variants share a common pathogenesis.

Methods: We stratified 51 patients with severe AD into extrinsic AD (n = 42) and intrinsic AD (n = 9) groups (with similar mean disease activity/SCORAD scores) and analyzed the molecular and cellular skin pathology of lesional and nonlesional intrinsic AD and extrinsic AD by using gene expression (real-time PCR) and immunohistochemistry.

Results: A significant correlation between IgE levels and SCORAD scores ($r = 0.76$, $P < 10^{-5}$) was found only in patients with extrinsic AD. Marked infiltrates of T cells and dendritic cells and corresponding epidermal alterations (keratin 16, Mki67, and S100A7/A8/A9) defined lesional skin of patients with both variants. However, higher activation of all inflammatory axes (including T_H2) was detected in patients with intrinsic AD,

particularly T_H17 and T_H22 cytokines. Positive correlations between T_H17-related molecules and SCORAD scores were only found in patients with intrinsic AD, whereas only patients with extrinsic AD showed positive correlations between SCORAD scores and T_H2 cytokine (IL-4 and IL-5) levels and negative correlations with differentiation products (loricrin and periplakin).

Conclusions: Although differences in T_H17 and T_H22 activation exist between patients with intrinsic AD and those with extrinsic AD, we identified common disease-defining features of T-cell activation, production of polarized cytokines, and keratinocyte responses to immune products. Our data indicate that a T_H2 bias is not the sole cause of high IgE levels in patients with extrinsic AD, with important implications for similar therapeutic interventions. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: Atopic dermatitis, eczema, extrinsic, intrinsic, IgE, T cell, human skin, keratinocytes, S100 proteins

Atopic dermatitis (AD) is a common inflammatory skin disease that together with asthma and allergic rhinitis forms the atopic triad.¹ Both AD and asthma are subtyped as extrinsic and intrinsic, representing approximately 80% and 20% of adult atopic patients, respectively. Extrinsic AD is characterized primarily by high serum IgE levels, as well as a personal and familial history of atopy and specific IgEs to food or aeroallergens. Intrinsic AD shares a similar clinical phenotype but exhibits normal serum IgE levels, absence of other atopic diseases, and lack of allergen-specific IgEs.^{2,3} Although peripheral eosinophilia can be seen in the setting of both types of AD, higher recruitment of eosinophils to inflamed tissues⁴ and prolonged eosinophil lifespan have been observed in patients with extrinsic AD.^{5,6}

Because the majority of patients with AD exhibit high IgE levels, an “allergic” IgE-mediated disease pathogenesis has been historically hypothesized. However, current models also associate AD with T-cell activation, particularly T_H2/T_H22 polarization, with a T_H1 component in patients with chronic AD and a possible contribution of T_H17.⁷ Thus high levels of the T_H2 cytokines IL-4 and IL-13 in AD skin lesions could influence immunoglobulin class-switching, promoting excessive IgE production.^{8,9} These cytokines have also been identified as inhibitors of epidermal differentiation and production of antimicrobial peptides.¹⁰⁻¹²

Inconsistent differences between intrinsic and extrinsic AD have been reported on a limited array of T_H1- and T_H2-related cytokines in PBMCs, lesional skin, and atopic patch test sites, as has the expression of FcεRI on antigen-presenting cells.¹³⁻¹⁸ Furthermore, prior comparisons did not explore the role of the more recently discovered T_H17 and T_H22 T cells.

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Abbreviations used

AD: Atopic dermatitis
 DC: Dendritic cell
 FLG: Filaggrin
 FOXP3: Forkhead box protein 3
 IHC: Immunohistochemistry
 OX40L: OX40 ligand
 RT-PCR: Real-time PCR
 TRAIL: TNF ligand superfamily 10

Thus the T-cell activation pathways and cytokine circuits in skin lesions of patients with intrinsic versus those with extrinsic AD have not been well defined, and a global analysis of the molecular and cellular skin pathology of intrinsic and extrinsic AD is unavailable. In this study we compared the skin structure, cellular infiltrates, and molecular markers between intrinsic and extrinsic AD. Our data identified common disease-defining features of intrinsic and extrinsic AD, including marked T-cell and dendritic cell (DC) skin infiltration and activation, that are associated with epidermal alterations (ie, hyperplasia and barrier abnormalities). Overall, we have found a higher immune activation in patients with intrinsic compared with extrinsic AD, particularly of the T_H17 and T_H22 immune axes. Additionally, both intrinsic and extrinsic AD lesions showed marked T_H2 activation (high IL-4/IL-13 expression), suggesting that a T_H2 bias is not the sole cause of high IgE levels in patients with extrinsic disease.

METHODS**Patient population**

For this study, we grouped several cohorts of patients with AD previously published by our group,^{7,19-23} as well as 3 new patients ($n = 51$). Chronic lesional (>72 hours duration with lichenification) and nonlesional (≥ 10 cm from active lesions) AD skin biopsy specimens and serum samples were collected for these studies under institutional review board–approved protocols. Patients were stratified into extrinsic (42 total; 23 male and 19 female patients; age, 15-81 years; mean age, 36.3 years) and intrinsic (9 total; 8 male patients and 1 female patient; age, 20-67 years; mean age, 40.3 years) AD categories. To define extrinsic and intrinsic status, we primarily used IgE levels, with values of greater than 200 kU/L defining extrinsic AD and values of less than 200 kU/L defining intrinsic AD. A summary of the demographics and clinical characteristics is presented in Table I and Table E1 in this article's Online Repository at www.jacionline.org. Serum IgE levels ranged between 26 and 93 kU/L (mean, 52.1 kU/L) in the intrinsic group and between 200 and 70,364 kU/L (mean, 3,577 kU/L) in the extrinsic group (reference range, 0-200 kU/L; $P = 1.14 \times 10^{-15}$). Eosinophil counts were significantly increased in the extrinsic group compared with the intrinsic group (extrinsic range, 0.30% to 13.60% [mean, 6%]; intrinsic range, 1.20% to 6.70% [mean, 2.9%]; reference range, 0% to 7%; $P = .006$). Scores on the SCORAD index, which is used to evaluate disease severity, were similar between the intrinsic and extrinsic patients (intrinsic range, 42-76 [mean, 54]; extrinsic range, 28-97.5 [mean, 53]; $P = .46$). Two patients with extrinsic AD were found to have filaggrin (FLG) mutations (1 heterozygous for the R501X allele and 1 heterozygous for the 2282del4 allele), whereas 19 extrinsic patients did not. However, 21 extrinsic patients predated our FLG genotyping studies, and mutation information was unavailable.^{19,21,24} No FLG gene mutations were found in the 9 patients with intrinsic AD. No associations between IgE status and age ($P = .49$, t test), sex, or family history were found ($P = .07$ and $P = 1$ respectively, Fisher exact test).

Skin biopsy specimens were not taken from skin that was clinically judged to be infected. No systemic or topical treatments were allowed for 4 or more

weeks before biopsy (see Table E1). Tissue samples were frozen in OCT medium for immunohistochemistry (IHC) and liquid nitrogen for RNA extraction. Histologic and real-time PCR (RT-PCR) analyses were previously performed on available nonlesional and lesional skin samples,^{7,19-23} with some additional analyses performed for the present study. Because of limited tissue availability, some analyses were only performed on a limited number of patients (see the Methods section in this article's Online Repository at www.jacionline.org).

IHC and RT-PCR

IHC was performed on cryostat tissue sections by using purified mouse anti-human mAbs (see Table E2 and the Methods section in this article's Online Repository at www.jacionline.org). Epidermal thickness was quantified, and positive cells per millimeter were counted manually by using computer-assisted image analysis software (ImageJ 1.42; National Institutes of Health, Bethesda, Md).^{7,19-23}

Sample preparation for RT-PCR

RNA was extracted for RT-PCR, which was performed with EZ-PCR Core Reagents (Life Technologies, Grand Island, NY), and custom primers were generated for analysis (see the primer list in the Methods section in this article's Online Repository).

Statistical analyses

The RT-PCR values normalized to human acidic ribosomal protein were transformed to the $\log_2$ scale; values of 0 were substituted with 20% of the minimum value observed for that gene within each study. Because data were gathered from multiple studies, the existence of a batch or study effect must be addressed, and data must be adjusted. Because some studies did not enroll patients with intrinsic AD, all available patients with extrinsic AD (defined by high IgE levels) have been used to estimate the batch/study effect, and these estimators have then been used to adjust the batch effect on all patients. Subsequently, only data from patients with chronic AD and known IgE status were retained for further analyses.^{7,19-23}

As in previous publications,^{7,19-23} both expression values (in $\log_2$) and cell counts were modeled by using a linear mixed-effects model, with tissue (lesional/non-lesional) and IgE category as the fixed effect and a random intercept for each patient. The comparisons of interest were tested by using contrasts. Some markers were excluded from the analyses because of sample size limitations (minimum sample size criteria for inclusion in analysis was as follows: $n = 4$ per group for statistical comparisons between groups and $n = 6$ for the correlation analyses). All analyses were carried out with statistical language R (www.R-project.org). Word clouds were created by using the homonym package R, with word sizes scaled to the $\log_2$ fold change of each comparison.

RESULTS

We studied a group of 51 patients, of whom 42 were classified as having extrinsic AD and 9 as having intrinsic AD. The disease severity was very similar among these groups (mean SCORAD score, 53 and 54 for extrinsic and intrinsic AD, respectively; $P = .46$; see patient characteristics in Tables I and E1). In the extrinsic AD group a significant correlation was found between increased IgE levels and higher disease activity, as detected by using the SCORAD score ($r = 0.76$, $P = 3.7 \times 10^{-7}$); such a correlation was not found in the intrinsic AD group (Fig 1).

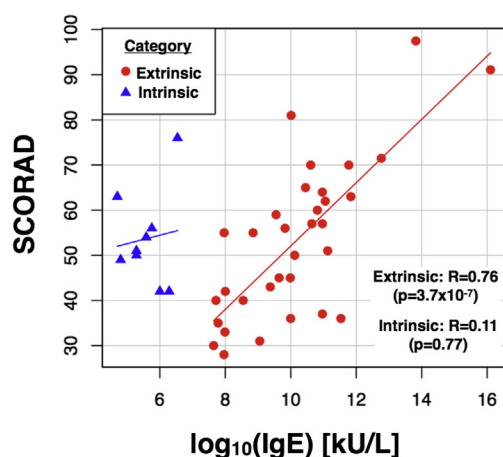
The cellular, molecular, and gene expression differences between the intrinsic and extrinsic AD transcriptomes were analyzed, defining the gene expression differences in lesional and nonlesional disease phenotypes of patients with extrinsic AD and those with intrinsic AD as the respective AD transcriptomes, as previously described (Figs 2-5).^{7,22,23}

TABLE I. Summary of clinical characteristics and demographics of patients with AD categorized as intrinsic AD by IgE levels of less than 200 kU/L and extrinsic AD with IgE levels of 200 kU/L or greater

	Intrinsic AD	Extrinsic AD	P value
IgE (kU/L)*	52.1 ± 22.7	3,576.2 ± 11,429	1.4×10^{-15}
SCORAD score*	54 ± 10.6	53 ± 17.3	.46
Eosinophils (%)*	2.9 ± 0.02	6 ± 0.03	.006
Total no. of patients	9	42	—
Sex (male/female; n)	8/1	23/19	.07
Family or personal history of atopy (positive/negative/unreported; n)	3/6/0	8/14/20	1

IgE levels and eosinophil counts differed significantly between the groups ($P = 1.4 \times 10^{-15}$ and $P = .006$, respectively), whereas the mean SCORAD score was very similar among the groups ($P = .46$).

*Mean ± SD.

**FIG 1.** Scatter plot of the correlation between IgE levels and SCORAD scores in extrinsic (red) and intrinsic (blue) AD categories. Lines represent linear regression within each group. Only patients with extrinsic AD exhibit a significant Pearson correlation between SCORAD scores and IgE levels ($R = 0.76$, $P = 3.7 \times 10^{-7}$), which is lacking in patients with intrinsic AD ($R = 0.11$, $P = .77$).

Marked epidermal hyperplasia and increased cellular infiltrates in lesional skin characterize both AD subtypes

Both intrinsic and extrinsic AD lesions are characterized by marked increases in T-cell numbers ($CD3^+$ and $CD8^+$ cells, $P < .005$ for both) and myeloid DCs ($CD11c^+$ cells, $P = .003$ for intrinsic AD and $P = .02$ for extrinsic AD) compared with non-lesional skin (Figs 2 and 3). Epidermal hyperplasia was detected to a similar extent in both variants based on measures of epidermal thickness ($P = .86$; Fig 3, A), MKi67 staining ($P = .16$; Fig 3, B), and mRNA expression of keratin 16 ($P = .65$; Fig 4, A). mRNA expression of terminal differentiation products was similar between both variants (see Fig E2 in this article's Online Repository at www.jacionline.org).

Additionally, both AD variants also demonstrate similar increases in DC subset numbers, although some differences were detected in the magnitude of T cells and DC infiltrates (Figs 2 and 3). Higher infiltrates of T cells ($CD3^+$ and $CD8^+$), myeloid DCs ($CD11c^+$), Langerhans cells ($CD1a^+$), and mature DCs ($CD83^+$) were detected in patients with intrinsic AD ($P < .05$ for all). Less

significant differences were observed for inflammatory dendritic epidermal cells ($Fc\epsilon RI^+$ and $CD206^+$), resident DCs ($CD1c^+$), and atopic DCs ($OX40$ ligand [$OX40L$] $^+$ and TNF ligand superfamily 10 [$TRAIL$] $^+$). Neutrophils (neutrophil elastase positive) were more abundant in patients with intrinsic AD ($P = .009$; Fig 3, M, and see Fig E1, B, in this article's Online Repository at www.jacionline.org). Eosinophil counts, as detected by using major basic protein (Fig 3, N, and see Fig E1, C), and plasmacytoid DC counts marked by blood dendritic cell antigen 2 (Fig 3, O) were greatly increased in patients with lesional extrinsic AD ($P = .08$ and $P = .008$, respectively) compared with values seen in those with intrinsic AD. Overall, larger cellular infiltrates were detected in patients with intrinsic AD (Figs 2 and 3 and see Fig E1).

More robust immune activation in patients with intrinsic AD

Intrinsic and extrinsic AD were both associated with significant increases between lesional and nonlesional AD in gene expression levels of T_H2 -defining (IL-10 and IL-13), T_H2 -defining (IL-22), and T_H1 -defining (interferon) cytokines. Levels of the IL-17/ T_H17 -related (IL-17, IL23p40, and CCL20; $P = .002$, $P = .05$, and $P = .004$, respectively) and T_H9 -related (IL-9) products were significantly increased only in lesional intrinsic AD skin. Moreover, when comparing lesional skin in patients with intrinsic and those with extrinsic AD, significant increases were detected in expression levels of genes related to inflammation (matrix metalloproteinase 12; $P = .006$), as well as T_H2 -related (CCL18, CCL22, thymic stromal lymphopoietin receptor, and OX40L), T_H22 -related (IL-22), interferon/ T_H1 -related (MX-1), T_H17 -related (IL-17 and CCL20), and regulatory T cell-related (forkhead box protein 3 [FOXP3], $P = .06$) molecules. IL-19 ($P = .12$) was the only cytokine with levels that were increased in extrinsic versus intrinsic lesional AD skin (Fig 4).

Overall, lesional skin in patients with intrinsic AD shows similar or higher T_H2 and T_H1 activity but significantly more robust T_H22 and T_H17 immune responses compared with lesional skin of patients with extrinsic AD. Patients with intrinsic AD showed a strong T_H17 molecular fingerprint in lesional skin (Figs 2-4 and see Fig E3, A and B, in this article's Online Repository at www.jacionline.org). Overall, nonlesional skin appeared very similar between the 2 groups, and only a small number of genes (OX40L, FOXP3, and IL-33; $P = .09$, $P = .08$, and $P = .08$, respectively) showed any appreciable differences between patients with intrinsic and those with extrinsic AD (Fig 4, L and DD, and see Fig E3, C and D).

Epidermal and T-cell pathway activation defines the AD transcriptome regardless of IgE status

To best visualize similarities and differences between extrinsic and intrinsic AD phenotypes (as determined by lesional versus nonlesional comparisons), we have created word clouds and scatter plots illustrating the magnitude of difference in protein (by IHC) and mRNA (by RT-PCR) levels (see the Methods section in this article's Online Repository for details). The word clouds are scaled proportionately, with numbers added (in black) to illustrate fold change differences. Significance is conveyed by color (Fig 5). The diameter of the circles in the scatter plots is proportionate to the relative fold changes and greater significance of the phenotype differences is emphasized by darker colors.

Overall, as shown in Fig 5, the intrinsic AD phenotype is characterized by both higher leukocyte cell counts and gene

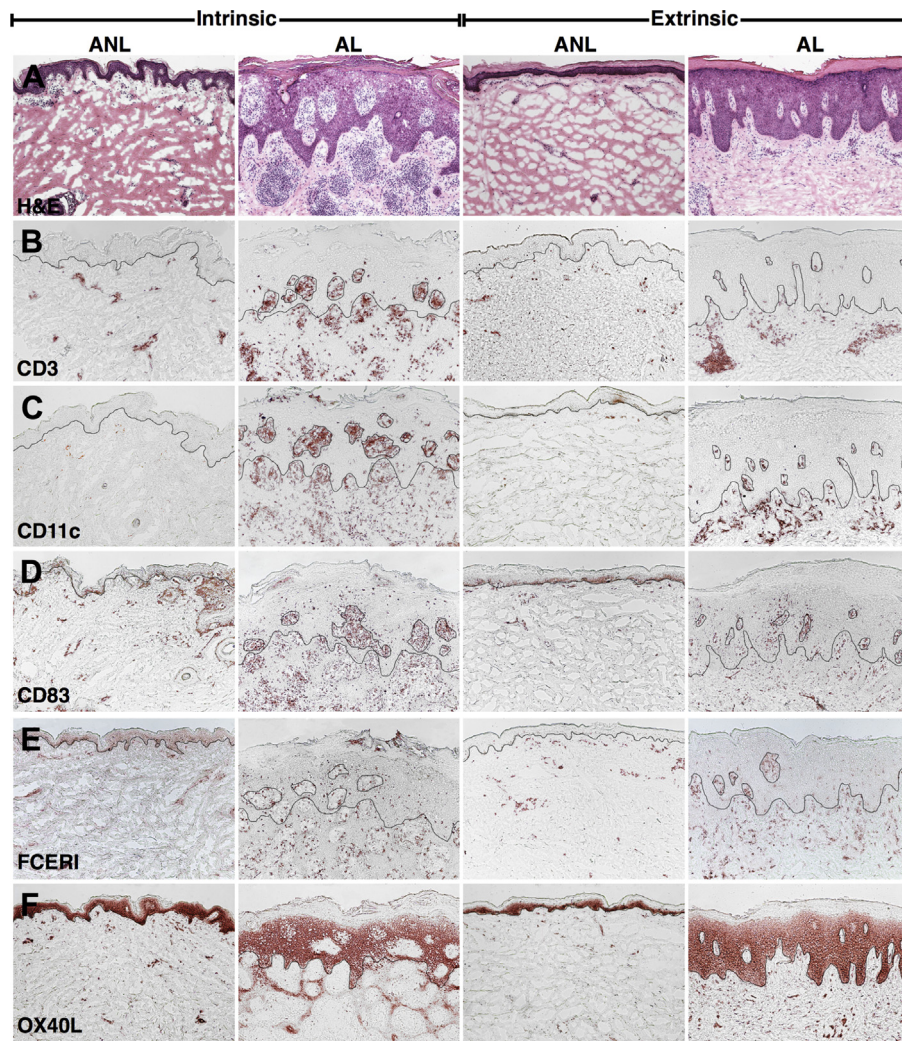


FIG 2. Representative hematoxylin and eosin (H&E) staining and IHC of lesional and nonlesional skin of patients with extrinsic and those with intrinsic AD. **A**, H&E staining demonstrates similar hyperplasia in extrinsic and intrinsic lesions. **B–F**, Large T-cell (CD3⁺), myeloid DC (CD11c⁺), mature DC (CD83⁺), inflammatory DC (FcεR1⁺), and atopic DC (OX40L⁺) infiltrates are evident in lesional skin of both groups (original magnification $\times 10$). AL, Lesional; ANL, nonlesional.

expression for a subset of inflammatory genes. However, only selected cellular infiltrates and genes showed significant differences in the intrinsic versus extrinsic AD phenotypes (Fig 5, C and F). Of particular interest are significant increases in the expression of IL-17A ($P = .002$), which are found only in the intrinsic AD transcriptome (Fig 5, F).

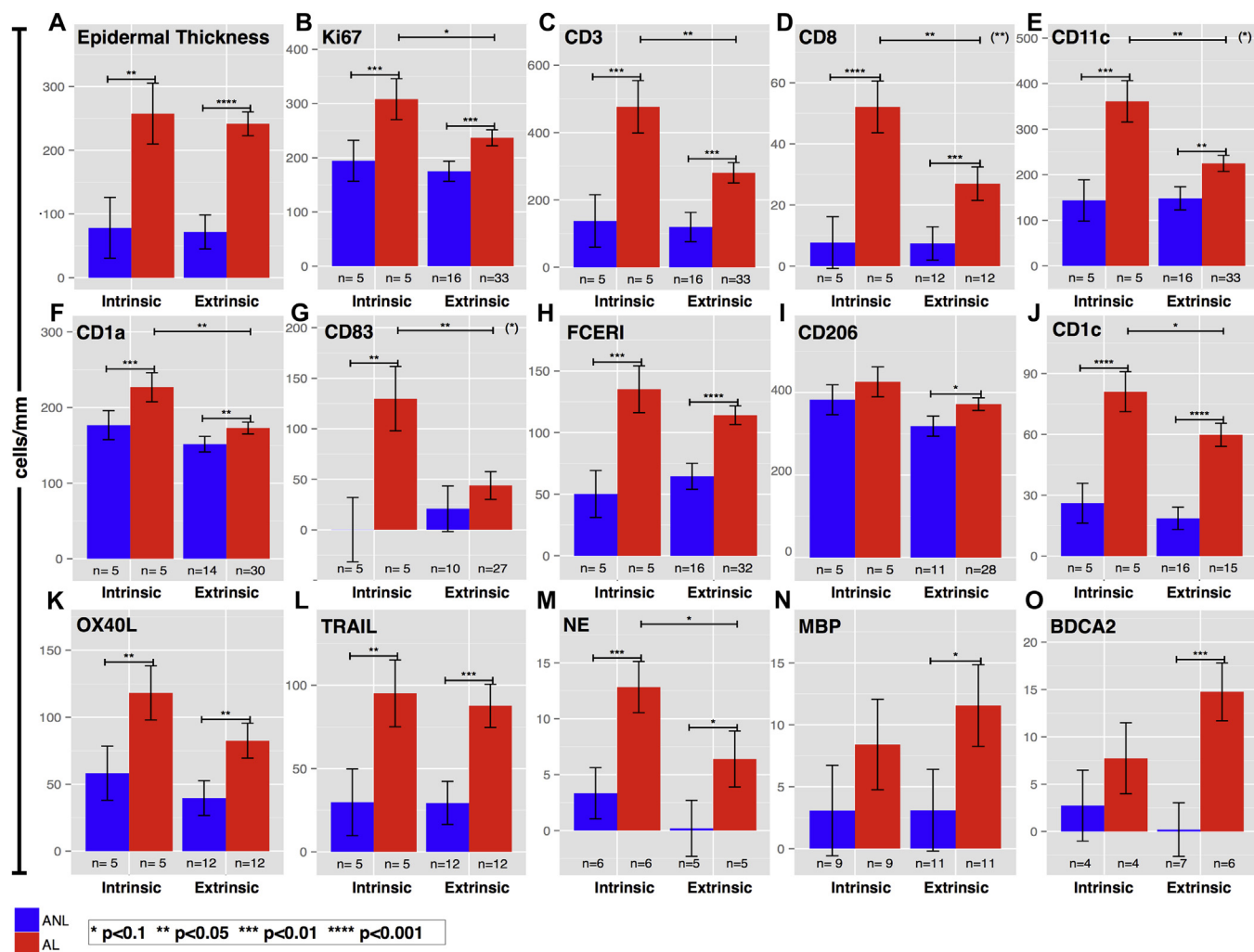
Both intrinsic and extrinsic AD phenotypes showed significant activation of mRNA gene expression of the S100 epidermal responses and the T_H1, T_H2, and T_H22 inflammatory pathways by means of RT-PCR. Contrary to our expectation, not only was there no T_H2 bias in the extrinsic AD group, but we actually found higher immune activation of all immune axes, including the T_H2 axis, in the intrinsic AD group (Fig 5, D and F).

Associations with disease activity in intrinsic and extrinsic AD

To correlate disease activity (determined by the SCORAD) with variables measured by using IHC and RT-PCR in lesional

skin, we used the Pearson correlation coefficient. In the intrinsic AD group, mRNA and protein expression of several inflammatory mediators in lesional tissues was correlated with the SCORAD score (Fig 6, A, and see Tables E3 and E4 in this article's Online Repository at www.jacionline.org). These included inflammatory mediators, such as IFN- α ($r = 0.81$, $P = .03$, $n = 7$), S100A12 (S100 Calcium-Binding Protein A12/Enrage; $r = 0.75$, $P = .05$, $n = 7$), matrix metalloproteinase 12 ($r = 0.66$, $P = .07$, $n = 8$), TRAIL ($r = 0.75$, $P = .05$, $n = 7$), and the T_H17-associated chemokine CCL20 ($r = 0.68$, $P = .09$, $n = 7$; Fig 6, A, and see Tables E3 and E4).

In the extrinsic AD group high correlation coefficients between SCORAD scores and lesional skin expression were noted for inflammatory mediators (ie, S100A12); T_H2-related cytokines, such as IL-4 ($r = 0.67$, $P = .07$, $n = 8$); and the T_H2-promoting cytokines IL-21 ($r = 0.67$, $P = .07$, $n = 8$)²⁵ and IL-19 ($r = 0.62$, $P = .09$, $n = 8$).^{26,27} Furthermore, a strong negative correlation was detected only in the extrinsic group between the SCORAD score and mRNA expression of terminal differentiation



genes, such as loricrin ($r = -0.59$, $P = .02$, $n = 16$) and periplakin ($r = -0.43$, $P = .09$, $n = 16$; Fig 6, B, and see Tables E3 and E4).

DISCUSSION

Although intrinsic AD represents a minority of patients with AD, it is important to clarify whether it represents a single disease spectrum along with extrinsic AD or whether different immune mechanisms underlie the 2 variants.

Numerous studies have tried to define differences and similarities in pathogenic mechanisms of extrinsic and intrinsic AD.^{2,4,14,16,28-30} Such studies have suggested common disease features of epidermal hyperplasia and increased infiltration of T cells and DC subsets but indicated a T_H2 bias and increased

eosinophil counts in patients with extrinsic AD.^{2,14,16,31} Although previous studies of T-cell polarization in skin lesions of patients with extrinsic and those with intrinsic AD have focused on T_H1 and T_H2 subsets/cytokines, activation of novel T-cell subsets, T_H22 and T_H17 T cells, might also be important for disease pathogenesis. While AD becomes increasingly recognized as associated with T_H22 T cells, the role of T_H17 T cells in patients with AD is still controversial.³²⁻³⁴ A few studies showed an increased T_H17 axis in patients with AD,^{35,36} but overall, the T_H17 pathway is much less activated in patients with AD compared with that seen in patients with psoriasis.²⁰ IL-17 and IL-22 cytokines derived from the newly recognized T-cell subsets have major effects on epidermal keratinocytes (ie, regulating the transcription of S100A7, S100A8, and S100A9 mRNAs in human keratinocytes).^{37,38} The onset of acute AD shows significantly higher

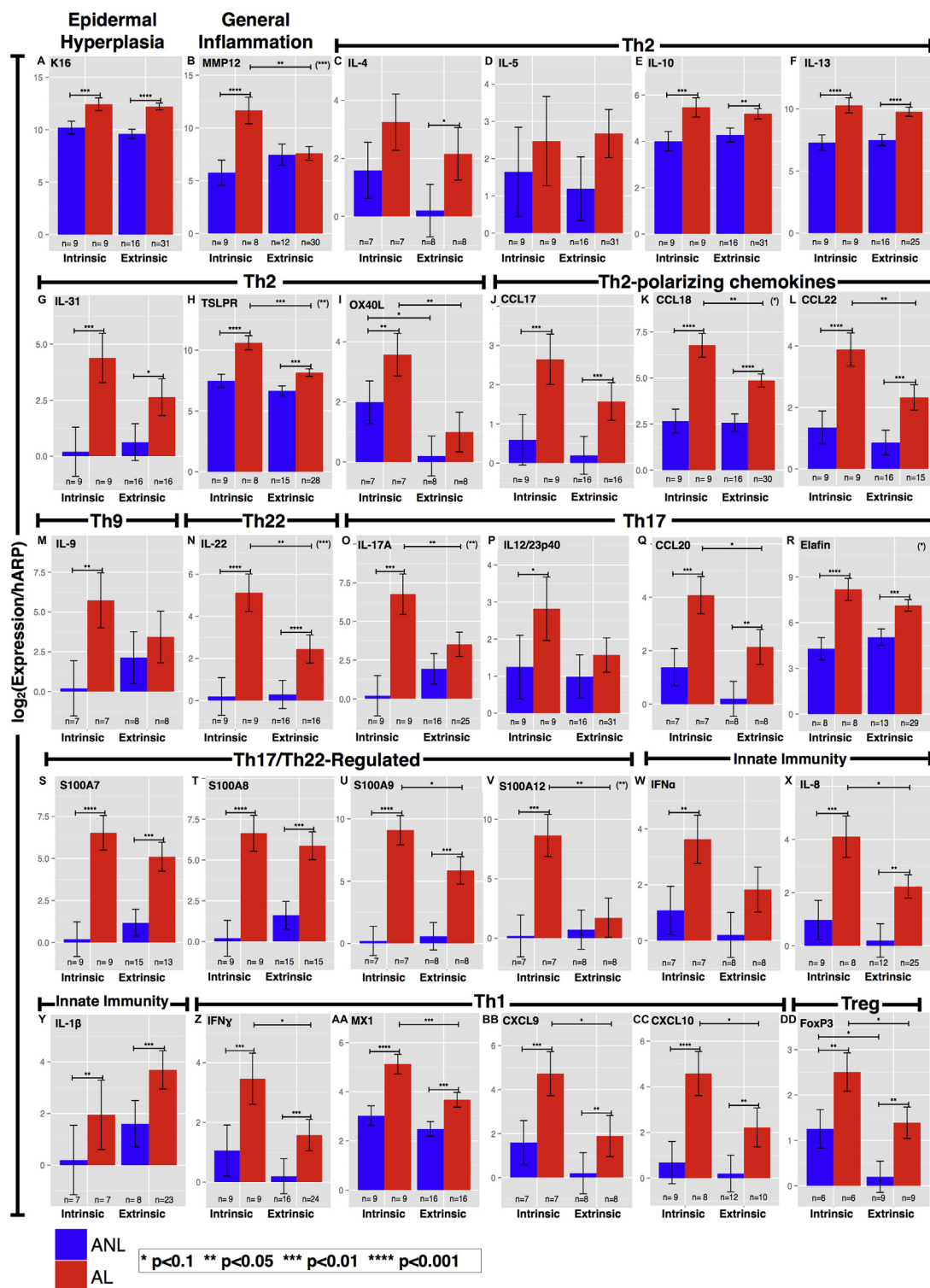
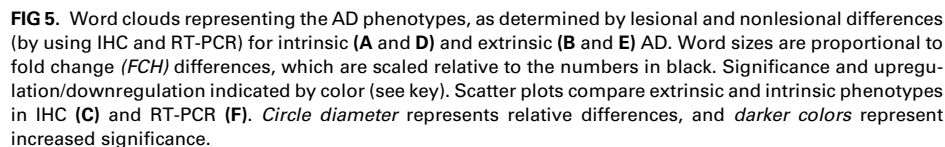


FIG 4. Bar plots of mRNA expression in lesional and nonlesional skin from patients with intrinsic and those with extrinsic AD. **A-DD**, Similar hyperplasia and activation of all inflammatory axes characterize lesional versus nonlesional skin in both variants. Intrinsic lesions exhibited more significant increases in markers of inflammation and T_H1 , T_H22 , T_H17 , and regulatory T (*Treg*) cells. Degree of significance between any comparisons is indicated if the P value is less than .1. Asterisks in parentheses (top right corners) represent significance of the interaction term of the ANOVA model; if present, the AD phenotypes greatly differ between the variants (means $\pm$ SEMs). * $P < .10$, ** $P < .05$, *** $P < .01$, and **** $P < .001$. AL, Lesional; ANL, nonlesional.



with extrinsic AD. S100A9 and S100A12 mRNA levels are higher in intrinsic versus extrinsic lesions, which might be explained by the higher expression of both IL-17 and IL-22 in intrinsic lesional skin and their potential synergistic effects on production of these S100s. Production of cathelicidin/LL-37 has also been reported to be lower in extrinsic compared with intrinsic skin lesions.^{13,43} Because LL-37 is strongly induced by IL-17 in human keratinocytes, the previously noted difference in LL-37 expression in patients with intrinsic AD might be explained by higher levels of IL-17. Additional evidence for higher IL-17 activation in intrinsic lesions is suggested by the mRNA expression of the IL-17-regulated CCL20 and Elafin, as well as greater numbers of neutrophils in patients with intrinsic AD, likely reflecting an increased T_H17/IL-17 axis in intrinsic disease. Epidermal hyperplasia, which was comparably increased in intrinsic and extrinsic skin lesions (Figs 3, A, and 4, A), could be directly stimulated by IL-22 overproduction in skin lesions; additionally, however, it could also be influenced by other cytokines, such as IL-19, IL-20, and IL-24.^{44,45} From the study of IL-17 blockade in patients with psoriasis,⁴⁶ it also appears that this cytokine can regulate keratinocyte hyperplasia, most likely through secondary

The hypothesized model of barrier inhibition through activation of T_H2 cytokines^{10,11,59,60} could be operative in both patients with extrinsic and those with intrinsic AD. However, our results noted strong correlations between disease activity/SCORAD scores and T_H2 cytokine levels (IL-4 and IL-5) paralleled by negative correlations with barrier products (ie, loricrin, periplakin, and FLG) only in patients with extrinsic AD (Fig 6, B). In contrast, patients with intrinsic AD demonstrated strong correlations between the SCORAD score and levels of T_H1/interferon-related genes (ie, IL-1 β and IFN- α) and the IL-17-related CCL20 chemokine (Fig 6, A). Thus in patients with intrinsic AD, there might be competing effects of T_H1, T_H17, and T_H2 cytokines on epidermal differentiation, so that cytokine effect models might differ in this disease variant. Emerging studies of IL-4 receptor (REGN668/clinical.trials.gov) and thymic stromal

lymphopoietin receptor blockade (MK8226-003/clinicaltrials.gov), which are being conducted in patients with both AD forms, might help clarify the physiologic effects of T_H2 cytokines on skin disease and associated barrier pathology.

We acknowledge a few inherent limitations of this study, including a retrospective design, several patient cohorts stratified for the extrinsic versus intrinsic analysis, and an unbalanced sample size. Nevertheless, our study defines molecular characteristics of both disease variants, with significant differences between patients with intrinsic and those with extrinsic AD. Our data, supported by the similarly effective therapeutic responses of patients with extrinsic and those with intrinsic AD to cyclosporin A, a broad T-cell suppressant,⁶¹ suggest that both AD forms are primarily T-cell driven. Beyond the T_H2 antagonism that could be used regardless of disease variant, the high expression of IL-17, IL-22, and IL-23/IL-12p40 suggests that use of other selective antagonists against these cytokines might have potential therapeutic benefits in patients with AD, as directed by similarities and differences between its extrinsic and intrinsic forms.

Clinical implications: Both extrinsic and intrinsic AD variants might be treated with T cell-targeted therapeutics or agents that modify keratinocyte responses.

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METHODS

Patients' characteristics and skin samples

For this study, we grouped several cohorts of AD patients previously published by our group^{E1-6} as well as three new patients (Total n=51). Lesional and non-lesional (≥ 10 cm from active lesions) skin biopsies and serum samples were collected from these studies under Institutional Review Board-approved protocols. Only chronic lesions were used for this study, differentiated from acute AD by duration >72 hours and with lichenification.^{E6} One cohort of patients^{E1-3} (n=18) underwent only lesional sampling (See Supplementary Table E1 in the Online Repository for breakdown of lesional and non-lesional tissue by cohort). Venous blood samples were taken from patients for serum IgE measurement (reference range: 0-200 kU/L), serum eosinophil levels (reference range: 0% to 7%) and DNA sequencing (see below). A summary of patient demographics and clinical characteristics may be found in Table 1 and Supplementary Table E1 (See Online Repository materials).

For this analysis, patients from these earlier studies were then stratified into intrinsic and extrinsic categories based on their IgE status. 42 patients (23 males, 19 females, age=15-81 years; mean age=36.3 years) were classified as extrinsic, determined by elevated IgE (range: 200-70364 kU/L, mean=3577 $\pm$ 11429 kU/L). 9 patients (8 males, 1 female, age=20-67 years; mean age=40.3 years) were classified as intrinsic, determined by normal IgE (range: 26-93 kU/L; mean=52 $\pm$ 23 kU/L; p=0.07). Serum eosinophil counts were increased in 8 of 42 extrinsic patients (range=0.30% - 13.60%, mean=6%) and 0 of 9 intrinsic patients (range=1.20%-6.70%, mean=2.9%; p=0.006). No associations between IgE status and: Age (p=0.49, t-test), Sex (p=0.07,

Fisher's Exact test), or Family history ($p=1$, Fisher's Exact test) were found.

The severity of AD was evaluated in all patients by using the Scoring of AD (SCORAD) index, as previously described⁽⁷⁾, and the SCORAD score was used to classify AD in each patient as mild (<25), moderate (25-50) or severe (>50) (maximum SCORAD score, 103), as previously defined.^{E8} The SCORAD index formula is as follows: $A/5 + 7B/2 + C$. In this formula A is defined as the extent of disease (percentage of BSA involvement, arrived at by application of the "rule of nines" applied to a front/back drawing of the patient), B is defined as the intensity (erythema, edema/papulation, excoriations, lichenification, oozing/crusts, and dryness), and C is defined as the subjective symptoms (daily pruritus and sleeplessness).^{E7} SCORAD index scores ranged from 28 to 97.5 (mean=53) in extrinsic patients and 42 to 76 (mean=54) in intrinsic patients ($p=0.46$).

Genotyping was previously performed for several key filaggrin (FLG) mutations (see below for methods). 2 patients with extrinsic AD were found to have FLG mutations (1 heterozygous for the R501X allele and 1 heterozygous for the 2282del4 allele); 19 extrinsic patients and all 9 intrinsic patients exhibited wild-type FLG. Filaggrin mutation analysis was not performed on 21 patients as they predated routine FLG mutation studies.

Skin biopsies were not taken from skin that was clinically judged as infected. No systemic or topical treatments were allowed within 4 weeks prior to biopsies. Tissue samples were frozen in OCT medium for Immunohistochemistry (IHC) and liquid nitrogen for RNA extraction. Histologic and real-time PCR (RT-PCR) analyses were previously performed^{E1-6} on available non-lesional and lesional skin samples with

additional analyses performed for purposes of this study. Due to limited tissue availability, some analyses were performed on a limited number of patients.

Immunohistochemistry

Cryostat tissue sections of all patients were stained with hematoxylin (Fisher, Fair Lawn, NJ) and eosin (Shandon, Pittsburgh, PA). Immunohistochemistry (IHC) was performed by using purified mouse anti-human monoclonal antibodies (Table E2). Biotin-labeled horse anti-mouse antibodies (Vector Laboratories, Burlingame, Calif) were amplified with avidin-biotin complex (Vector Laboratories) and developed with chromogen 3-amino-9-ethylcarbazole (Sigma-Aldrich, St Louis, MO). All immunostained sections were photographed and analyzed with ImageJ software to measure epidermal thickness and cell counts per millimeter (ImageJ 1.42; National Institutes of Health, Bethesda, MD). Previous cell counts from earlier studies^{E1-6} were combined with additional staining performed for this study. Variable numbers of patients were analyzed for each stain for reasons explained above; for clarification, Figure 3 (representing IHC quantification) includes respective *n* values below each plot for the intrinsic and extrinsic groups.

Genotyping of Filaggrin Variants

FLG genotyping methodology has been described in the methods sections for previously published studies from which our cohorts were derived.^{E4-6} Genomic DNA samples were isolated from whole blood from all patients included in the study and genotyped for two of the most common filaggrin null mutations, R501X and 2282del4, using TaqMan allelic discrimination assays as previously described^{E9,E10}, with minor modifications. Briefly, TaqMan assays using Universal PCR Master Mix in 10-ml

reaction volumes were run under standard TaqMan conditions on a ViiA7 Real-Time PCR System (Life Technologies, Grand Island, NY). For R501X, primers were used at 300 nM, probes at 200 nM, and template DNA at 0.625 ng.^{E9} For 2282del4, the forward-1 and reverse primers were used at 1000 nM, the forward-2 primer at 200 nM, probes at 300 nM, and template DNA at 5 ng.^{E11}

RT-PCR analysis

RT-PCR analysis was gathered from previously collected data.^{E1-6} Sample size varied per gene, and respective *n* values are depicted underneath the plots in Figure 4 and Supplementary Figure 2 in the online repository. The TaqMan RT-PCR reaction was performed with EZ-PCR Core Reagents (Life Technologies), according to the manufacturer's directions. The primers and probes for K16 (assay ID Hs00955088_g1), IL-31 (assay ID Hs01098710_m1), IL-32 (assay ID Hs01098710_m1), IL-33 (assay ID Hs00369211_m1), IL-17A (assay ID Hs00174383_m1), IL-9 (assay ID Hs00914237_m1), IL23(p19) (assay ID Hs00372324_m1), IL23(p40) (assay ID Hs0001011518_m1), IL22 (assay ID Hs01574154_m1), IL-4 (assay ID Hs00174122_m1), IL13 (assay ID Hs99999038_m1), IL-10 (assay ID Hs00961622_m1), IL-5 (assay ID Hs00174200_m1), CCL5 (assay ID Hs00174575_m1), CCL26 (assay ID Hs00171146_m1), CCL17 (assay ID Hs00171074_m1), CCL18 (assay ID Hs00268113_m1), CCL22 (assay ID Hs01574247_m1), CCL2 (assay ID Hs00234140_m1), CCL13 (assay ID Hs00234646_m1), CCL11 (assay ID Hs99999025_m1), IFN γ (assay ID Hs00989291_m1), IFN α 1 (assay ID Hs00256882_m1), MX1 (assay ID Hs00895608_m1), IL-8 (assay ID Hs00174103_m1), IL-22 (Hs01574154_m1), IL-1 β

93 (assay ID Hs01555413_m1), Foxp3 (assay ID Hs00203958_m1), CXCL9 (assay ID
 94 Hs00171065_m1), CXCL10 (assay ID Hs00171042_m1), CXCL11 (assay ID
 95 Hs00171138_m1), CCL20 (assay ID Hs01011368_m1), OX40L (assay ID
 96 Hs00182411_m1), TRAIL (assay ID Hs00234356_m1), TSLPR (assay ID
 97 Hs00845692_m1), PI3/Elafin (assay ID Hs00160066_m1), LCN2 (assay ID
 98 Hs00856927_m1), FLG (assay ID Hs00856927_g1), LOR (assay ID Hs01894962_s1),
 99 CDSN (assay ID Hs00169911_m1), S100A7 (assay ID Hs00161488_m1), S100A8
 100 (assay ID Hs00374263_m1), S100A9 (assay ID Hs00171086_m1), and S100A12
 101 (assay ID Hs00942835_m1) were designed by Life Technologies.

102 The human acidic ribosomal protein (hARP) gene, a housekeeping gene, was
 103 used to normalize each sample and each gene. The primer sequences used were
 104 generated with the Primer Express algorithm (Applied Biosystems, Foster City, Calif),
 105 version 1.0, by using published genetic sequences (National Center for Biotechnology
 106 Information–PubMed), as follows: hARP forward CGCTGCTGAACATGCTCAA, hARP
 107 reverse TGTCGAACACCTGCTGGATG, and hARP probe 6-FAM-
 108 TCCCCCTTCTCCTTTGGGCTGG-TAMRA (Gene Bank accession no. NM-001002).
 109 The data were analyzed and quantified by using the software provided with the Applied
 110 Biosystems PRISM 7700 (Sequence Detection Systems, version 1.7).

111 **Statistical analyses**

112 The RT-PCR values normalized to hARP were transformed to the $\log_2$ scale (values of 0
 113 were substituted with 20% of the minimum value observed for that gene within each
 114 study). Since data were gathered from multiple studies, the existence of a batch, or
 115 study effect must be addressed and data adjusted. As some studies did not enroll

intrinsic patients, all available extrinsic patients (defined by high IgE status) have been used to estimate the batch/study effect and we have adjusted the values accordingly. Subsequently, only data from patients with chronic AD and known IgE status were retained for further analyses.^{E1-6}

As in previous publications^{E1-6}, both expression values (in $\log_2$) and cell counts were modeled by a linear mixed-effects model, with Tissue (LS/NL) and IgE Category as the fixed effect and a random intercept for each patient. The comparisons of interest were tested using contrasts. Some markers were excluded from the analyses due to sample size limitations (minimum sample size criteria for inclusion in analysis was: $n=4$ per group for statistical comparisons between groups, and $n=6$ for the correlation analyses). Analyses were performed using a mix of paired (lesional/non-lesional) and unpaired (lesional only); when available, paired tissues were taken into consideration in all statistical calculations. All analyses were carried out using statistical language R (www.R-project.org). Word clouds were created using the homonym package R with word sizes scaled by $\log_2$ -fold change in the different comparisons.

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Intrinsic

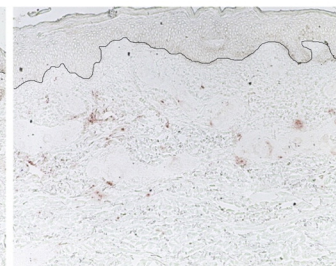
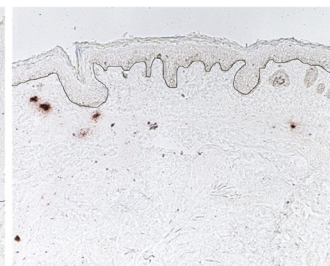
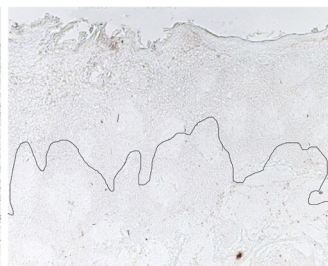
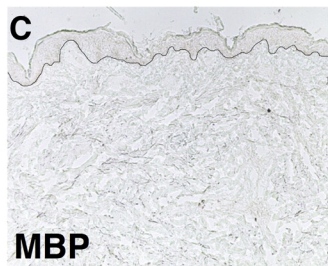
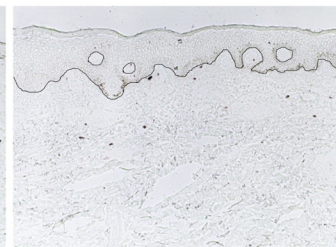
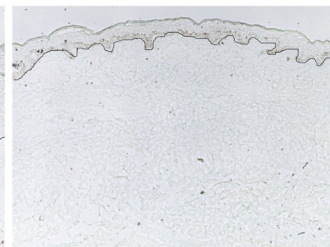
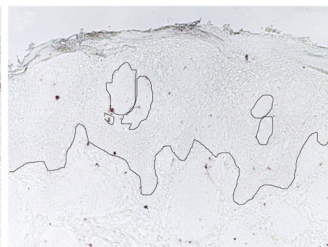
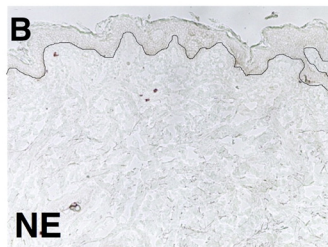
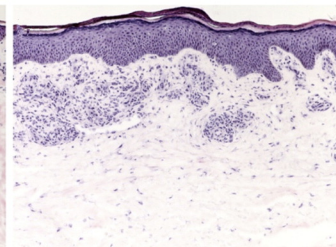
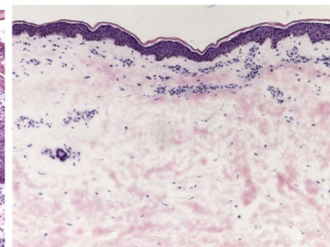
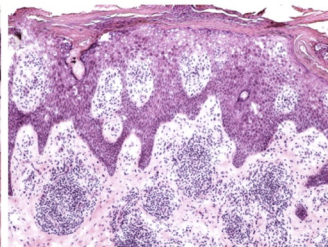
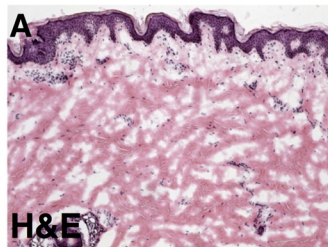
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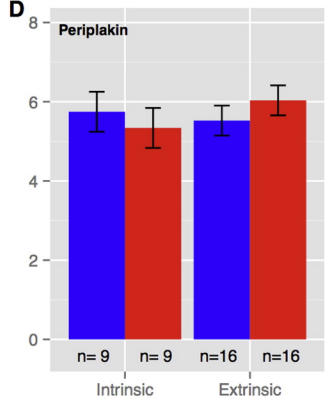
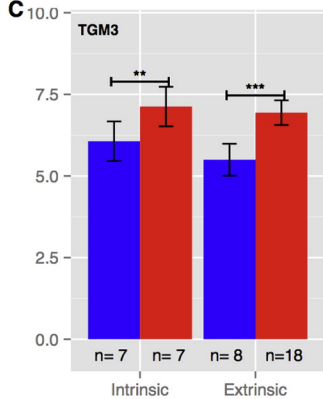
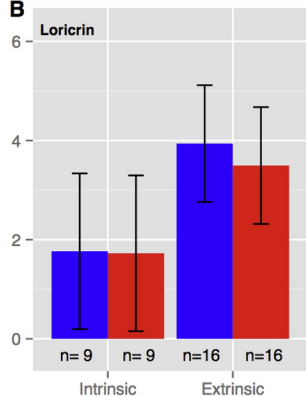
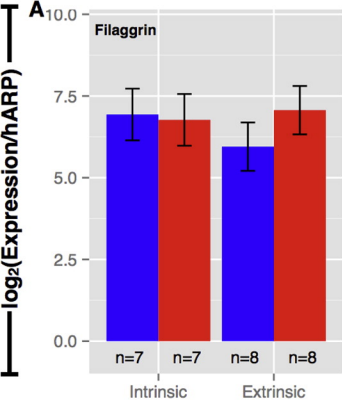
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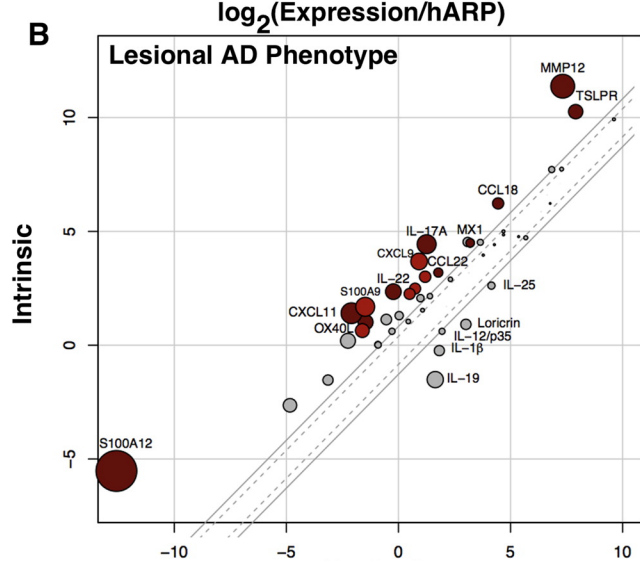
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Lesional AD Phenotype
(Intrinsic vs. Extrinsic)



Non-Lesional AD Phenotype
(Intrinsic vs. Extrinsic)

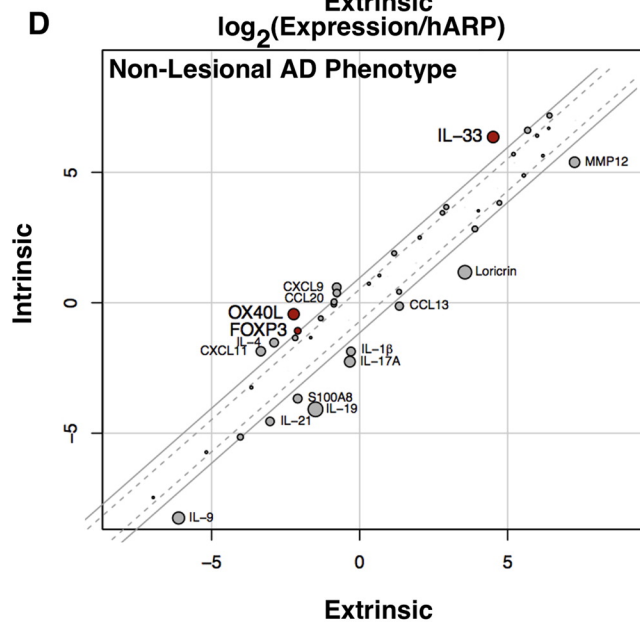
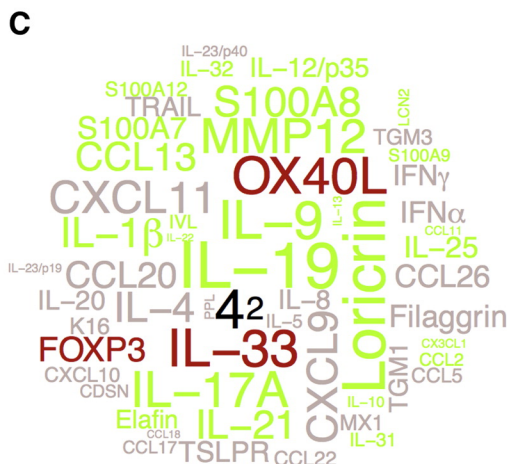


Table E1: Patient Characteristics of Intrinsic and Extrinsic Atopic Dermatitis patients**A) Individual Characteristics**

Patient	Age (y)	Gender	Personal and/or Family history of atopy ^a	SCORAD ^b	IgE ^c	Intrinsic or Extrinsic ^d	Eos. Count ^e
1	39	F	No	37	2000	Extrinsic	6%
2	48	M	No	51	39	Intrinsic	4.30%
3	36	M	Yes	36	1021	Extrinsic	4.40%
4	26	M	No	33	254	Extrinsic	7.60%
5	30	M	Yes	57	2000	Extrinsic	2.10%
6	50	M	Yes	56	907	Extrinsic	3.70%
7	42	M	No	55	461	Extrinsic	1.30%
8	43	F	No	28	249	Extrinsic	2.60%
9	23	M	Yes	76	93	Intrinsic	6.70%
10	38	M	No	64	2000	Extrinsic	1.30%
11	41	M	Yes	70	1562	Extrinsic	5.90%
12	36	F	No	71.5	6965	Extrinsic	3.30%
13	81	M		91.1	70364	Extrinsic	5.70%
14	15	F	No	81	1032	Extrinsic	13.60%
15	38	F	No	97.5	14474	Extrinsic	11.30%
16	21	F				Extrinsic	
17	78	M				Extrinsic	
18	17	M			6000	Extrinsic	
19	18	F		70	3500	Extrinsic	
20	20	F				Extrinsic	
21	22	M		55	250	Extrinsic	
22	27	M		40	210	Extrinsic	
23	23	M		35	220	Extrinsic	
24	35	M		65	1400	Extrinsic	
25	20	F			200	Extrinsic	
26	25	F				Extrinsic	
27	20	M		57	1600	Extrinsic	
28	24	F		30	200	Extrinsic	
29	30	M		45	800	Extrinsic	
30	60	M		60	1800	Extrinsic	
31	29	M			250	Extrinsic	
32	27	M			300	Extrinsic	
33	26	M			200	Extrinsic	
34	62	M	Yes	50	1112	Extrinsic	10.30%
35	37	F	Yes	62	2126	Extrinsic	6.40%
36	39	F		42	256	Extrinsic	7.40%
37	59	M	No	56	54	Intrinsic	1.40%
38	45	M	No	42	64	Intrinsic	2.50%
39	47	F	No	45	1014	Extrinsic	8.00%
40	26	M	Yes	42	78	Intrinsic	2.20%
41	42	M	Yes	63	26	Intrinsic	2.30%

42	67	M	No	54	48	Intrinsic	3.90%
43	33	F	No	50	39	Intrinsic	1.20%
44	45	F	No	63	3652	Extrinsic	0.30%
45	52	F	No	40	374	Extrinsic	4.70%
46	20	M	No	49	27.9	Intrinsic	1.20%
47	33	F	Yes	43	663	Extrinsic	5.30%
48	26	F	Yes	59	750	Extrinsic	6.90%
49	60	M	No	51	2238	Extrinsic	7.50%
50	58	M	No	31	531	Extrinsic	4.90%
51	28	F	No	36	2962	Extrinsic	2.30%

a: Family history defined as history of allergic rhinitis and/or hay fever, atopic dermatitis, and/or asthma in parent and/or sibling

b: SCORing of Atopic Dermatitis index (see Methods)

c: Reference range, 0-160kU/L

d: Patient defined as intrinsic if IgE fell within the reference range; patient defined as extrinsic if IgE exceeded upper limit of reference range

e: Reference range, 0-7% serum eosinophil count

B) Cohort Groupings from Previous Studies

Patient Cohorts from Previous Studies				
Study	Guttman-Yassky et. al. 2007^{E1} Guttman-Yassky et. al. 2008^{E2} Guttman-Yassky et. al. 2009^{E3}	Suárez-Fariñas et. al. 2011^{E5}	Gittler et. al. 2012^{E6}	Unpublished
n	18	15	15	3
Extrinsic / Intrinsic (n)	18 / 0	13 / 2	8 / 7	3 / 0
Lesional / Non-lesional (n)	18 / 0	15 / 15	15 / 15	3 / 3

Table E2. Antibodies used for immunohistochemical analysis

Antibody	Vendor	Clone	Isotype	Dilution
Keratin 16 (K16)	Sigma Aldrich	K8.12	IgG1	1:40
Ki67	Santa Cruz	MIB-1	IgG1	1:100
CD3	BD Biosciences	SK7	IgG1	1:100
CD8	Abcam	144B	IgG1	1:25
CD11c	BD Biosciences	B-ly6	IgG1	1:100
CD1c/BDCA-1	Miltenyi Biotec	AD5-8E7	IgG2a	1:100
CD1a	Abcam	CD1a007	IgG1	1:25
CD1b	BD Biosciences	M-T101	IgG1	1:25
FcεRI	Thermo Scientific	CRA1 (AER37)	IgG2b	1:100
TRAIL	R&D Systems	75402.11	IgG1	1:50
OX40L	R&D Systems	159403	IgG1	1:50
BDCA-2	Miltenyi Biotec	AC144	IgG1	1:5
CD206	Genetex	15-2	IgG1	1:100
CD83	BD Biosciences	HB15e	IgG2	1:20
DC-LAMP	Fisher Scientific	104.G4	IgG1	1:50
Major Basic Protein	Thermo Scientific	BMK13	IgG1	1:15
Neutrophil Elastase	Dako	NP57	IgG1	1:200

Tryptase	Abcam	AA1	IgG1	1:100
Langerin	Immunotech	DCGM4	IgG1	1:100
CD11b	BD Pharmingen	ICRF44	IgG1	1:10
CD205	RM Steinman lab, Rockefeller University	MG38	IgG2b	1:100
CD86	Bio Legend	IT2.2	IgG2b	1:50
iNOS	Santa Cruz	N20	IgG	1:20
DC-SIGN	BD Pharmingen	DCN46	IgG2b	1:50

Table E3: Cellular and Molecular Correlations (Pearson coefficient) with Disease Activity (SCORAD) in Lesional AD skin

Variable (mRNA in black; Cell counts in blue)	R (Extrinsic)	p-value (Extrinsic)	n	R (Intrinsic)	p-value (Intrinsic)	n
SCORAD	1.00	0.00	33	1.00	0.00	9
log(IgE)	0.76	0.00	33	0.11	0.77	9
TRAIL	0.68	0.07	8	0.75	0.05	7
IL-21	0.67	0.07	8	-0.19	0.68	7
IL-4	0.67	0.07	8	0.65	0.12	7
S100A12	0.63	0.09	8	0.75	0.05	7
IL-19	0.62	0.10	8	0.21	0.65	7
CXCL9	0.58	0.13	8	-0.16	0.73	7
IFNa	0.57	0.14	8	0.81	0.03	7
CXCL11	0.56	0.15	8	-0.08	0.86	7
IgE	0.52	0.00	33	0.22	0.57	9
OX40L	0.44	0.28	8	-0.32	0.48	7
IL-9	0.43	0.29	8	-0.37	0.42	7
MMP12	0.39	0.06	24	0.66	0.07	8
TGM3	0.39	0.15	15	0.47	0.28	7
S100A9	0.38	0.35	8	-0.05	0.91	7
IL-5	0.37	0.07	25	0.02	0.96	9
MX1	0.36	0.17	16	-0.11	0.77	9
CCL20	0.36	0.38	8	0.68	0.10	7
IL-20	0.35	0.40	8	0.26	0.57	7
Elafin	0.32	0.13	23	0.42	0.30	8
IL-17A	0.32	0.14	23	-0.27	0.48	9
TRAIL	0.31	0.32	12	0.64	0.24	5
S100A8	0.31	0.27	15	-0.03	0.94	9
CD8	0.30	0.34	12	0.71	0.18	5
CD206	0.30	0.21	19	-0.69	0.20	5
IL-22	0.30	0.26	16	-0.32	0.40	9
TGM1	0.30	0.18	22	0.04	0.92	9
IL-32	0.27	0.51	8	0.20	0.67	7
CCL2	0.26	0.39	13	0.17	0.68	8
CXCL10	0.25	0.48	10	-0.08	0.85	8
IL-8	0.25	0.27	22	-0.25	0.55	8
OX40L	0.24	0.46	12	0.01	0.98	5
IL-31	0.23	0.39	16	-0.36	0.34	9
CCL22	0.22	0.44	15	-0.06	0.88	9
IVL	0.20	0.67	7	-	-	-

Variable (mRNA in black; Cell counts in blue)	R (Extrinsic)	p-value (Extrinsic)	n	R (Intrinsic)	p-value (Intrinsic)	n
BDCA-2	0.19	0.71	6	0.74	0.26	4
K16	0.17	0.42	25	-0.17	0.66	9
Epidermal Thickness	0.10	0.65	24	-0.41	0.49	5
S100A7	0.09	0.76	13	0.01	0.98	9
IL-10	0.09	0.68	25	0.43	0.24	9
CD1a	0.08	0.72	21	0.98	0.00	5
CD11c	0.08	0.71	24	-0.41	0.49	5
Thickness-ST	0.08	0.71	24	-0.24	0.70	5
CD3	0.07	0.75	24	0.13	0.83	5
CCL17	0.07	0.80	16	-0.03	0.93	9
CCL11	0.06	0.81	16	-0.31	0.42	9
CCL13	0.06	0.84	15	0.01	0.99	8
FcER1	0.04	0.84	24	0.49	0.40	5
CDSN	0.04	0.85	23	0.27	0.48	9
IL-1B	0.03	0.90	17	0.69	0.08	7
CD83	-0.02	0.93	18	-0.30	0.62	5
Ki67	-0.03	0.90	24	-0.27	0.67	5
CCL18	-0.03	0.88	24	-0.29	0.45	9
IFN-g	-0.04	0.88	22	-0.21	0.59	9
TSLPR	-0.06	0.78	22	0.29	0.49	8
FOXP3	-0.08	0.84	9	-0.37	0.47	6
IL-12p35	-0.10	0.63	24	-0.04	0.95	5
IL-33	-0.11	0.80	8	0.14	0.77	7
LCN2	-0.11	0.70	15	0.34	0.42	8
IL-23p19	-0.11	0.60	25	0.00	1.00	9
CCL26	-0.12	0.77	8	-0.23	0.62	7
CD1b	-0.13	0.65	15	-	-	-
CX3CL1	-0.13	0.64	15	0.72	0.04	8
IL-13	-0.14	0.54	23	0.41	0.27	9
Tryptase	-0.19	0.57	11	-0.68	0.21	5
CD1c	-0.19	0.49	15	-0.61	0.27	5
CCL5	-0.21	0.40	18	0.12	0.78	8
MBP	-0.22	0.52	11	-0.27	0.49	9
IL-23p40	-0.23	0.62	7	-	-	-
IL-25	-0.27	0.52	8	-	-	-
IL-23p40	-0.28	0.17	25	-0.27	0.49	9
Filaggrin	-0.31	0.45	8	0.72	0.07	7

Variable (mRNA in black; Cell counts in blue)	R (Extrinsic)	p-value (Extrinsic)	n	R (Intrinsic)	p-value (Intrinsic)	n
PPL	-0.44	0.09	16	-0.58	0.10	9
DC-LAMP	-0.56	0.12	9	-0.62	0.57	3
Loricrin	-0.59	0.02	16	-0.04	0.91	9
Neutrophil Elastase	-0.64	0.25	5	-0.12	0.82	6

Table E4: Cellular and Molecular Correlations (Pearson Coefficient) with Disease Activity (SCORAD) in Non-Lesional AD skin

Variable (mRNA in black; Cell counts in blue)	R (Extrinsic)	p-value (Extrinsic)	n	R (Intrinsic)	p-value (Intrinsic)	n
SCORAD	1.00	0.00	24	1.00	0.00	9
BDCA-2	0.85	0.01	7	0.98	0.02	4
CXCL11	0.48	0.23	8	-0.09	0.85	7
CCL20	0.47	0.24	8	0.19	0.69	7
IL-20	0.47	0.24	8	0.54	0.22	7
CD3	0.46	0.07	16	0.32	0.60	5
TGM3	0.45	0.26	8	0.27	0.56	7
TRAIL	0.39	0.34	8	0.36	0.43	7
CD1a	0.39	0.17	14	0.54	0.35	5
IL-19	0.38	0.35	8	0.30	0.51	7
FcER1	0.31	0.25	16	0.43	0.47	5
MMP12	0.30	0.35	12	0.61	0.08	9
IL-4	0.27	0.52	8	-0.69	0.08	7
IFNa	0.22	0.60	8	0.74	0.06	7
OX40L	0.20	0.63	8	0.53	0.22	7
Tryptase	0.17	0.62	11	0.23	0.71	5
CD8	0.15	0.64	12	-0.08	0.90	5
CXCL9	0.08	0.84	8	-0.23	0.62	7
Filaggrin	0.06	0.89	8	0.40	0.37	7
Loricrin	0.06	0.84	16	-0.07	0.86	9
CCL17	0.05	0.86	16	0.03	0.95	9
IL-33	0.02	0.96	8	0.07	0.89	7
IL-1B	0.00	0.99	8	0.61	0.15	7
IL-8	-0.01	0.99	12	0.53	0.14	9
IL-23p19	-0.01	0.98	16	-0.16	0.69	9
Neutrophil Elastase	-0.01	0.99	5	0.21	0.69	6
CCL22	-0.02	0.94	16	0.18	0.65	9
K16	-0.04	0.87	16	0.22	0.57	9
IL-21	-0.06	0.89	8	-0.13	0.78	7
CX3CL1	-0.06	0.82	15	-0.22	0.56	9
CXCL10	-0.07	0.84	12	0.20	0.60	9
IL-13	-0.07	0.80	16	0.03	0.94	9
MX1	-0.08	0.78	16	0.16	0.68	9

Variable (mRNA in black; Cell counts in blue)	R (Extrinsic)	p-value (Extrinsic)	n	R (Intrinsic)	p-value (Intrinsic)	n
Ki67	-0.08	0.77	16	-0.66	0.22	5
IFN-g	-0.10	0.72	16	0.13	0.73	9
LCN2	-0.12	0.71	13	0.09	0.82	9
PPL	-0.12	0.67	16	-0.07	0.85	9
IL-32	-0.12	0.78	8	0.34	0.45	7
CCL26	-0.12	0.77	8	0.16	0.73	7
CCL2	-0.14	0.62	15	0.08	0.84	9
CDSN	-0.15	0.58	16	-0.02	0.96	9
log-IgE	-0.15	0.48	24	0.20	0.60	9
CD206	-0.15	0.65	11	0.36	0.55	5
CCL13	-0.16	0.63	12	-0.33	0.39	9
S100A9	-0.16	0.71	8	0.46	0.30	7
CCL18	-0.17	0.54	16	0.08	0.83	9
IL-23p40	-0.18	0.50	16	-0.36	0.34	9
CCL11	-0.20	0.46	16	-0.32	0.40	9
IgE	-0.22	0.31	24	0.29	0.45	9
TSLPR	-0.26	0.35	15	0.33	0.38	9
CD1c	-0.27	0.32	16	-0.13	0.84	5
TGM1	-0.28	0.32	15	0.14	0.71	9
IL-10	-0.28	0.30	16	0.08	0.83	9
S100A12	-0.28	0.50	8	0.26	0.57	7
IL-5	-0.29	0.27	16	0.01	0.98	9
CCL5	-0.32	0.29	13	-0.13	0.75	9
IL-12p35	-0.35	0.20	15	-0.23	0.70	5
IL-9	-0.35	0.40	8	0.18	0.70	7
IL-22	-0.37	0.16	16	-0.03	0.93	9
Elafin	-0.41	0.17	13	0.13	0.75	8
IL-17A	-0.42	0.11	16	0.00	1.00	9
S100A7	-0.43	0.11	15	0.34	0.37	9
S100A8	-0.45	0.09	15	0.25	0.52	9
IL-31	-0.53	0.03	16	0.09	0.82	9
MBP	-0.57	0.07	11	-0.18	0.64	9
FOXP3	-0.61	0.08	9	0.17	0.74	6
DC-LAMP	-0.65	0.24	5	0.98	0.12	3
Thickness-ST	-0.66	0.01	16	0.08	0.90	5
TRAIL	-0.68	0.01	12	0.13	0.83	5

Variable (mRNA in black; Cell counts in blue)	R (Extrinsic)	p-value (Extrinsic)	n	R (Intrinsic)	p-value (Intrinsic)	n
Epidermal Thickness	-0.73	0.00	16	-0.07	0.92	5
CD83	-0.76	0.01	10	-0.25	0.68	5
OX40L	-0.77	0.00	12	0.12	0.85	5
CD11c	-0.94	0.00	16	-0.02	0.97	5