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Progression of acute-to-chronic atopic dermatitis is associated with quantitative rather than qualitative changes in cytokine responses

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Author Contributions

L.C.T., J.E.G., and S.W. designed the study. D.S. performed all clinical assessments. S.W. and S.G. assisted with recruitment and clinical assessments. U.W. performed histopathological analyses. E.R. and F.T. contributed with clinical samples and/or preparing the library for the RNA-seq experiments. L.C.T. and E.R. conducted the quality control and processed the RNA-seq data. L.C.T., W.R.S., A.S., J.C., M.T.P., and M.H. conducted the bioinformatics analysis of the processed data. J.S., M.K.S., C.Z., R.U., X.X., A.C.B., J.E.G. contributed to the immunostaining and the siRNA knock-down experiment. L.C.T., U.W., P.W.H., J.M.K., E.M., J.E.G., and S.W., contributed to the biological inference of the analysis results. B.E.P.W. contributed to the keratinocytes, and M.K.S. and R.U. contributed to the cytokine stimulated experiments. L.C.T., J.E.G., and S.W. wrote the manuscript.

Conflict of interest

All other authors declare no relevant conflicts of interest.

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Abstract

Background—While multiple studies have assessed molecular changes in chronic atopic dermatitis (AD) lesions, little is known about the transition from acute to chronic disease stages, and the factors and mechanisms that shape chronic inflammatory activity.

Objectives—We sought to assess the global transcriptome changes that characterize the progression from acute to chronic stages of AD.

Methods—We analyzed transcriptome changes in paired non-lesional skin, acute and chronic AD lesions from 11 patients and 38 healthy controls by RNA-seq, and conducted *in vivo* and histological assays to evaluate findings.

Results—Our data demonstrate that ~74% of the genes dysregulated in acute lesions remain or are further dysregulated in chronic lesions, whereas only 34% of genes dysregulated in chronic lesions are altered already in the acute stage. Non-lesional AD skin exhibited enrichment of TNF, Th1, Th2, and Th17 response genes. Acute lesions showed marked dendritic cell signatures and a prominent enrichment of Th1, Th2 and Th17 responses, along with increased IL-36 and TSLP expression, which were further heightened in chronic lesions. In addition, genes involved in skin barrier repair, keratinocyte proliferation, wound healing and negative regulation of T cell activation showed a significant dysregulation in the chronic versus acute comparison. Furthermore, our data show progressive changes in vasculature and maturation of dendritic cell subsets with chronicity, with FOXK1 acting as immune regulator.

Conclusions—Our results show that the changes accompanying the transition from non-lesional to acute to chronic inflammation in AD are quantitative rather than qualitative, with chronic AD having heightened Th2, Th1, Th17, and IL36 responses and skin barrier repair mechanisms. These findings provide novel insights and highlight underappreciated pathways in AD pathogenesis that may be amenable to therapeutic targeting.

Capsule summary

Our study provides a comprehensive view of the pathologic processes that take place in acute to chronic progression for AD. Changes from acute to chronic AD are quantitative rather than qualitative in terms of shifts in Th2, Th22, Th1 and Th17 responses.

Keywords

Atopic dermatitis; RNA-seq; non-lesional; chronic AD; acute AD

I. Introduction

Atopic dermatitis (AD) is one of the most common inflammatory skin disorders affecting up to 20% of children and 10% of adults in high-income countries, with a worldwide prevalence of approximately 8% [1]. While its peak incidence is in childhood, adult AD is common, comprising both chronic persistent and/or relapsing-remitting as well as new-onset

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disease [2, 3]. The pathophysiology of AD is still not completely understood, but it is believed to be driven by epidermal barrier disruption, activation of specific T-cell subsets, and dysbiosis of the commensal skin microbiome [4]. The disease's hallmark clinical sign is eczematous lesions, which may present (sub-)acutely (diffuse scaly erythematous patches and oozing papulovesicles) or chronically (scaly patches and plaques with excoriation and lichenification). Histologically, acute lesions are characterized by spongiosis/edema between epidermal keratinocytes, mild to absent thickening of the epidermis, and infiltration with inflammatory cells along with degranulated mast cells; while chronic lesions show a marked thickening of the epidermis, less pronounced epidermal spongiosis, and a heavier mononuclear inflammatory infiltrate [5]. Acute and chronic histopathologic changes exist on a continuum. Patients with moderate-to-severe AD can experience acute and chronic lesions simultaneously, and the distinction between acute and chronic stages is not trivial, since clinical and histopathological features often overlap in lesions [6]. It is yet unclear whether acute and chronic stages are driven by different molecular mechanisms or alternatively represent a different magnitude of inflammatory response. One study that investigated the differences between "bona fide" acute and chronic lesions, in a cohort of ten patients using microarray gene expression profiling and quantitative RTPCR, found that acute lesions displayed an increase in *IL22*, *IL32* and *S100A7–9* along with type 2 cytokines including *IL4* and *IL31*; all of these, other than *IL4,* showed progressively greater expression in chronic lesions [7]. In contrast, an upregulation of Th1 response genes including *MX1* and *CXCL9–11*, were only seen in chronic lesions, suggesting that acute inflammation in AD is driven by type 2 cytokines, while chronic stages immunological mechanisms also involve Th1 responses. So far, these observations have not been replicated. Our group has previously conducted a large scale and deep molecular transcriptomic profiling for AD using RNA-seq [8]. Here, we analyzed acute versus chronic lesions in a subset of 11 AD patients, and by combining RNA-seq technology and paired design, we were able to provide novel insights with enhanced resolution on the mechanisms involved in acute to chronic AD progression. Further, we unraveled FOXK1 as a potential regulatory factor, responsible for differentiating the inflammatory responses in acute versus chronic AD.

II. Methods

Skin biopsies and RNA-seq

Intrapersonal acute, chronic (>72 hrs duration) and non-lesional (≥ 10 cm from active lesions) skin biopsies were collected from 11 consented Caucasian patients with moderate-to-severe AD, along with biopsies from 38 healthy volunteers included in a previous skin transcriptome study [8] under an Institutional Review Board-approved protocol (A110/12) (Suppl Table 1). In all patients, biopsies were taken from the upper arms (flexural side). No systemic or topical treatments were allowed for ≥4 weeks prior to biopsies. All patients used in this study met the following criteria for acute skin lesions: a) new lesions of <72 hours duration, as previously defined[7]; b) lack of skin lichenification; c) lack of histopathological signs of chronic eczema such as acanthosis, parakeratosis, and regenerative hyperplasia, as defined by epidermal thickness ≤150μm (H&E), and basal or confluent supra-basal Keratin 16 (K16) positivity. Patients were instructed to present at our department within 72 hours when noticing new lesions on previously unaffected skin but not in case of flares of

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preexisting chronic lesions. All clinical assessments were done by the same investigator. A total of 5 patients were excluded after histopathological examination of biopsies due to signs of chronic eczema (marked acanthosis, parakeratosis, hyperplasia)

After sampling skin tissue specimen were immediately stored in PAXgene® Tissue Containers (PreAnalytiX, Hombrechtikon, Switzerland) at −80°C until isolation of total RNA with the AllPrep DNA/RNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturers protocols. Preceding RNA isolation skin specimens were disrupted using innuSPEED Lysis Tubes W (1.4 – 1.6 mm steel beads & 3.5 mm ceramic beads) (Analytik Jena, Jena, Germany) in a SpeedMill Plus (3× 1min intervals) (Analytik Jena) together with 600µl of RLT-Plus-Buffer (Qiagen) and QIAshredder spin-columns (Qiagen). Quality control on concentration and integrity of the isolated RNA was performed with the Qubit 2.0 Fluorometer (Qubit® RNA HS Assay) (LifeTechnologies, Carlsbad, CA) and the 2200 Tape Station (R6K ScreenTape Assay) (Agilent, Santa Clara, CA) following the manufacturer's instructions. Only RNA samples with a concentration of >50ng/µl, an OD260/280 ≥1.8 and a RNA integrity number (RIN) >7 were included in subsequent library preparation and sequencing.

RNA samples were prepared for sequencing using the Illumina Truseq® Stranded total RNA Protocol in combination with the RiboZero rRNA removal Kit, and sequenced on the HiSeq2500 in pools of 10 samples with 2×125bp, producing paired-end reads according to the manufacturer's protocol (Illumina, San Diego, CA).

Filaggrin genotyping

In all patients, the 4 filaggrin loss-of-function variants that are most common in the German population (R501X, 2282del4, R2447X, and S3247X) were typed as described previously[8].

RNA-seq processing

All the data processing and analyzes were conducted under the Linux environment, and R was used for all the statistical analyzes. RNA-seq reads were first mapped to the human reference genome (b37) using STAR [9], and the number of uniquely mapped reads for each gene was counted using HTSeq [10]. Only genes with an average ≥1 read/sample were used in our analysis. The trimmed mean of M-values normalization method (TMM) was used to normalize the read count across samples [11], and we applied voom transformation to model the mean-variance relationship of the expression data [12]. We conducted differential expression analysis between different conditions using an empirical Bayes linear model as implemented in the limma package [13], controlling for individual specific effect (for the non-lesional vs. chronic AD, the non-lesional vs. acute AD, and the acute vs. chronic AD skin comparisons) and gender effect (for the other comparisons); False discovery rate (FDR) ≤10% and |log2 Fold Change (FC)| ≥1 were used to declare significance.

Cell culture, cytokines stimulations, RNA interference

N/TERTs, an immortalized keratinocytes cell line, was grown in KC-SFM medium (ThermoFisher #17005–042) with 30µg/ml bovine pituitary extract, 0.2ng/ml epidermal growth factor, and 0.3mM calcium chloride in 48-well plates and treated with small

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interfering RNAs (siRNAs) targeting FOXK1(Accell #E-032790–00-0010) and Non-target control siRNAs (Accell #D-001910–01-20) according to Dharmacon Accell siRNA protocol. Cell were either unstimulated or stimulated with the cytokines IL-4 (10ng/ml, R&D #204-IL-010), IL-10(10ng/ml, R&D #217-IL-005), IL-13(10ng/ml, R&D #213-ILB005) for 24h respectively.

RNA isolation and qRT-PCR

RNA was isolated using Qiagen RNeasy plus kit (Cat#74136). Reversed transcription was performed using High Capacity cDNA Transcription kit (ThermoFisher #4368813). qRT-PCR was performed using a 7900HT Fast Real-time PCR system (Thermor Fisher) with Taqman Universal PCR Master Mix (Thermo Fisher #4304437) and Taqman primers (Thermor Fisher: *FOXK1* Hs01595620_m1, *RPLP0* Hs00420895_gH, *CCL5* Hs99999048_m1, *IL32* Hs00992441_m1, *IL4R* Hs00965056_m1.

Immunohistochemistry and immunofluorescence

For immunohistochemistry, FFPE human skin biopsy specimens (independent of the RNA-seq samples) on slides were heated for 30 minutes at 65°C, rehydrated, epitope retrieved, blocked and incubated with primary antibody against FOXK1(ATLAS ANTIBODIES #HPA017998) overnight at 4°C. Slides were washed, incubated with secondary antibody, developed with diaminobenzidine and counterstained with hematoxylin. Secondary antibody used for immunofluorescence was from (Life technologies, Alexa Flour 488).

III. Results

We performed RNA-seq on biopsies from non-lesional and paired acute and chronic lesions, enabling us to conduct robust analysis of differences and potential progression from non-lesional to acute, to chronic stages of inflammation. We were able to profile 31,207 genes with an average of at least 1 read/sample. Notably, the top three principal components were not able to separate acute from chronic AD (Figure 1a). A previous microarray-based study employing less stringent criteria (i.e. $|\log_2$Fold Change (FC)$| \geq 0.585$ and p-value ≤ 0.01) [7] identified 47 up- and 96- down regulated genes. Using the same criteria, we found 197 up- and 233- down-regulated genes when comparing acute with chronic AD (Suppl Table 2). When corrected for multiple testing, our RNA-seq data identified 42 statistically significant ($|\log_2$ FC$| \geq 1$ and false discovery rate, FDR $\leq$ 10%) genes (29 up and 13 down in chronic AD when compared with acute AD; Suppl Table 3), including genes involved in epidermal differentiation (*S100A8* and *S100A9*) and response to epidermal barrier stress (*KRT16*, *KRT6B*), as well as members of the CXC chemokine (*CXCL1, CXCL6)*, TNF (*TNIP3*) and IL-10 cytokine family (*IL19*, *IL20*). Significantly, the majority of these genes overlap with the 15 genes that show a progressive expression pattern from non-lesional to acute to chronic AD (i.e. significantly up-regulated in each of the comparisons) (Figure 1b). Indeed, genes that were dysregulated in the acute AD vs. non-lesional comparison (609 up-regulated and 537 down-regulated) show a modest but consistent shift in their expression levels in the acute vs. chronic AD comparison, indicating the progression of the transcriptomic changes with development of chronicity (Figure 2a). Notably, our results reveal that most of the changes in acute AD are also present in chronic AD, as ~74% of the genes dysregulated in

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acute AD are also differentially expressed in chronic AD when compared with non-lesional skin (Suppl Table 3). On the contrary, only around 34% of the genes dysregulated in chronic AD are also differentially expressed in acute AD. We identified a set of genes that are statistically differentially expressed only between chronic and acute lesions, while showing little changes between acute vs. non-lesional AD (Figure 2b–d). These include genes that participate in skin barrier maintenance and repair (*HRNR*), IL-19, IL-20 [14–16], immunoglobulin production (*IGHG1*, *IGLC2*, *IGKC*), inhibition of CD4 + T cell activation (*CLEC3A*) [17], modulation of T-cell responses (*TNFAIP3*) (Schuijs MJ Science 2015; Vroman H J Allergy Clin Immunol. 2018), wound healing (CXCL1, CXCL6) (Griffith JW 2014), and tryptophan metabolism (*KYNU*) [18]. It is noteworthy that the immunoglobulins as a group have been identified as differentially expressed in chronic lesional skin of psoriasis and AD [8]; additionally, *KYNU*, the L-kynureninase, which regulates tryptophan metabolism, can serve as an immune response modulator for chronic inflammatory conditions [18]. No major differences were seen between FLG mutation carriers and non-carriers.

We then used the molecular profiles to provide a deeper insight into the changes in cellular compositions during disease progression, by comparing the control skin versus the non-lesional, acute, and chronic lesional skin of AD patients. By utilizing an *in silico* technique to study the expression signatures for different human cell types [19], we identified a significant trend of increasing infiltration of immune cells (i.e. dendritic; Th1, Th2, pro B-cells) and endothelial cells during the acute-to-chronic AD progression (Figure 3). The increase in a type-2 expression signature (Figure 4a) in chronic AD corresponded with the heightened immune response and the more pronounced epidermal hyperplasia/growth signaling in chronic AD. The presence of dendritic cell signatures in acute AD skin is of interest, and could correspond to the crucial role they play in type-2 response induction in this early inflammatory disease stage. Vasculature is known to play an important role in the pathophysiology of AD, as AD lesions are characterized by activated endothelial cells and their interactions with T cells are important for leukocyte trafficking into inflamed AD skin [20]. Thus, these findings align with the gradual increase in endothelial cell signature we observed in progression to chronic AD (Figure 3).

To better understand the cytokine expression and cytokine responses in acute vs. chronic AD, we compared the mRNA expression of the major cytokines across our dataset, as well as how well each skin type aligns with the cytokine response signatures (the down-stream responses) in keratinocytes. Using unbiased hierarchical clustering, we determined the grouping of the expression patterns of different cytokine families across acute and chronic AD, with the type 2 family (in particular, *IL13*) grouping with IL10 family cytokines (*IL19*, *IL20*, *IL22*), IL1/IL36 family (*IL36A*), and *TSLP* (Figure 4a). In concordance with our previous finding, we did not detect expression of *IL4* in acute AD skin. In addition to *IL19* and *IL22*, both *IL36G* and *TSLP* show progressive expression profiles from acute to chronic AD skin ($p=8.6\times10^{-4}$ and $p=2.6\times10^{-3}$, respectively). Assessing the down-stream cytokine responses [8] using healthy control skin as a reference, we observed that non-lesional AD skin already has enriched TNF, IFN, Th2, and Th17 response gene expression, and these cytokine response “burdens” become more pronounced in lesional AD (Figure 4b). Notably, chronic AD exhibits heightened inflammatory responses for all inflammatory signals that we measured when compared to acute AD (Figure 5). We also observed a large variation in

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these signatures within the chronic or acute AD stage, consistent with the clinical and molecular heterogeneity of AD. By correlating the genome-wide fold change between chronic vs acute versus the effect size during cytokine stimulation, we further consolidated the above findings, demonstrating that chronic AD is typified by dysregulated gene responses to IL17, IL13, TNF, and IFN, as well as an elevated response to IL36 (Supplementary Figure 1).

To understand the transcriptional regulatory network that differentiates acute from chronic AD we screened for transcription factor (TF) binding motif enrichment within the promoter regions of the genes that are significantly up-regulated in chronic AD (compared to non-lesional skin) but not differentially expressed in acute AD. Only the binding sites of FOXK1, a transcription factor that modulates developmental and cell differentiation processes [21–23], were found to be significantly enriched ($p=1.5\times10^{-6}$; FDR=9.5×10^{-4}). While *FOXK1* mRNA expression was neither dysregulated in non-lesional nor lesional AD, its protein expression was increased and showed prominent nuclear localization in epidermal keratinocytes in lesional skin, particularly in chronic lesions (Figure 6a). We next investigated the effect of FOXK1 on differentially expressed genes (in chronic AD) that were predicted to have FOXK1 binding sites (i.e. *IL4R*, *CCL5*, *IL32*). While *IL4R* was not affected, *CCL5*, *IL32*, and *FOXK1* itself were significantly induced upon stimulation with different type 2 associated cytokines, including IL-4, IL-10, and IL13, which we have previously outlined as the most prominent Th2 cytokines in AD [8]. Notably, under IL-13 induction we observed a significant effect upon FOXK1 knockdown, which led to increased expression of *CCL5* and *IL32*, suggesting that FOXK1 has a role as a negative immune regulator in chronic AD (Figure 6b).

IV. Discussion

Clinically, AD presents with eczematous lesions in different acuity stages. Acute and chronic lesions are often found in the same individual, often overlap, and clinically are sometimes difficult to distinguish [24]. Histologically, acute and chronic AD have fairly distinctive features. For instance, acute AD lesions exhibit spongiosis with mild to moderate acanthosis in addition to a superficial perivascular infiltrate of lymphocytes and macrophages. Mast cells show degranulation [25], and occasionally eosinophils may be present [26]. With increased chronicity, usually first noted in clinically subacute lesions, the acanthosis becomes more prominent, which can take on psoriasis-like features. In addition, the number of blood vessels increases [27]. However, the processes and mechanisms that are involved in the transition from acute to chronic AD are still not completely understood.

Only a limited number of studies have attempted to address the molecular differences between acute and chronic AD. The most comprehensive study on this subject was a microarray transcriptomic study on 10 patients with paired acute and chronic AD biopsy samples. This study in addition measured selected cytokines and chemokines using quantitative RT-PCR[7]. Based on the results, the authors suggested that acute AD is primarily triggered through action of IL-22, with smaller contributions from IL-17 and IFN-γ, accompanied by Th2 cytokines including IL-4/IL-13 and IL-31. In chronic lesions expression of Th2 and Th22 cytokines further increased, and higher activation of Th1 and

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Th17 responses was observed, accompanied by increase in epidermal growth and thickness [7]. To enable comparison with previous studies, we used the same clinical and histopathological criteria for differentiating acute from chronic AD. To further reduce variability, all patients were instructed to present at our department within 72 hours when noticing new lesions on previously unaffected skin, but not in case of flares of preexisting chronic lesions. All clinical assessments were done by the same investigator, and all biopsies were taken from the same anatomical region. Using higher resolution RNA-seq, our study highlights a more comprehensive and nuanced picture illustrating the continuum of the AD progression. Specifically, our results confirm that rather than a qualitative shift in the cytokine network and activated immune axes in chronic AD, all the major Th1, Th2, and Th17 responses are progressively heightened from non-lesional AD to acute and then chronic lesions (Figure 4b). This is also evident by our finding that there is not a clear distinction between the cytokine responses in acute AD vs. chronic AD, and frequently the acute lesions show biphasic responses suggesting an ongoing transition towards the chronic phase. Furthermore, it is striking that 74% of genes dysregulated in acute AD are also differentially expressed in chronic AD. In contrast, only 34% of DEGs found in chronic lesions (versus non-lesional skin) were altered in acute lesions. DEGs found only in chronic but not acute lesions have annotated functions in particular in “skin barrier maintenance and repair”, “wound healing”, “modulation of T-cell responses” and “immunoglobulin production”. This suggests that the main distinction between acute and chronic AD in terms of the cytokine network activation is quantitative rather than qualitative in nature, but that there are additional specific features associated with perpetuation and chronicity of inflammation.

IL-22 has been suggested to play a major role particularly in the early phases of AD [7, 28, 29]. IL-22 belongs to the IL-20 family of cytokines [30] and was not the only prominent IL-20 family member we observed to be upregulated in both acute and chronic AD lesions. Interestingly, in our study other IL-20 family members including IL-19, IL-20, IL-24, and IL-26 also showed prominent increases in both acute and chronic AD lesions to similar degree. This could provide an explanation for the limited clinical improvement seen with anti-IL-22 treatment in AD [31], as it is likely that in the presence of IL-22 inhibition, other members of this family are able to compensate given their similarities and overlapping receptor usage.

Both TSLP and the IL-36 family of cytokines are expressed predominantly by epithelial cells [32, 33], and act on a number of cells including epithelial cells and immune cells [34]. TSLP induces dendritic cells (DCs) to express OX40 ligand (OX40L), which binds to the OX40L receptor on T cells to stimulate the production of Th2 cytokines [35]. IL-36 cytokines do not act directly on T cells but instead can stimulate maturation and function of DCs and through them drive T cell proliferation [36], thereby propagating and amplifying immune responses in skin. The shift to chronic AD is accompanied by an increase in TSLP and IL-36 activity, in accordance with their established role in amplifying and sustaining inflammatory reactions in the skin, suggesting that TSLP, its inducers, and the IL-36 cytokine axis might be potential therapeutic targets in AD. The potential for success in this strategy is evident, as therapeutic targeting of TSLP in 113 patients with AD showed promising responses in a phase II clinical trial [37]. Current clinical studies are ongoing, focusing on the OX-40

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inhibition in atopic dermatitis. While no data yet exists on anti-IL-36 treatment in AD, agents targeting this inflammatory axis have already been tested with promising results in pustular psoriasis [38]. Therefore, the contribution of IL-36 family cytokines in the pathogenesis of chronic AD and their therapeutic relevance should be assessed in the near future.

The histologic features of acute and chronic AD, as outlined above, are well established. Our findings are consistent with acute to chronic AD histologic changes. They highlight a progressive enrichment in gene signatures, attributed to endothelial cells, Th1 and Th2 cells being more prominent in chronic AD compared to acute AD (Figure 3). By contrast, with increased chronicity we observed decreased signatures for myeloid, lymphoid progenitor, and hematopoietic stem cells, likely reflecting the increased activation and maturation of resident immune cells during chronic AD. We also observed an increased pro B-cell signature in chronic lesions. Whereas regulatory B cells have been shown to be decreased in patients with AD [39], B cell counts are increased in the skin and blood of patients with AD; this may correlate with increased IgE levels [40], which is a prominent feature of AD [4]. Thus, our inflammatory cell signatures align closely with what has been described clinically for AD.

The mechanisms involved in regulating chronic inflammation in AD are not well established. Our data provide some novel insights into these mechanisms. We observed a prominent activation of signatures related to skin barrier repair and wound healing, and we identified two transcription factors that may have relevance for the regulation of inflammatory responses. These factors include FOXE1, which was one of a limited group of genes showing progressive changes from inflamed acute to chronic AD (Suppl Table 2), and FOXK1, which did not show changes in its mRNA expression but had enriched binding sites amongst genes having differential expression in chronic AD lesions. FOXK1 is a transcription factor that modulates developmental and cell differentiation process [21–23]. Not much is known about the role of FOXK1 in inflammatory responses, but it has been shown to regulate *CCL2* expression and recruitment of tumor associated macrophages [41]. Our data suggest that in chronic AD it has an immune-regulatory role that is fairly specific to IL-13 responses, which we have previously shown to be the dominant inflammatory response in AD [8]. Little is known about the role of FOXE1 in epidermal biology or inflammatory responses, with most of the data on this transcription factor being from cancer biology, where it has been shown to have anti-proliferative effects [42].

Taken together, our study provides a high-resolution view of the pathologic processes that take place during the acute to chronic conversion that occurs in AD. Our findings suggest that the changes from acute to chronic AD are quantitative rather than qualitative in terms of shifts in Th2, Th22, Th1 and Th17 responses, with additional features developing only in chronic inflammation. We also illustrate the elevated IL-36 responses in the chronic phase of AD. These findings provide novel insights into the pathogenesis of atopic dermatitis and highlight previously understudied pathways in AD pathogenesis that may be amenable to future therapeutic targeting. They need to be corroborated, however, by investigation in larger well assembled cohorts.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AD	atopic dermatitis
FDR	False discovery rate
FC	Fold Change

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Key messages

- Our study highlights the continuum of AD progression from acute to chronic stages with progressively heightened inflammatory responses for all major immune axes.
- Our study identified 42 significantly dysregulated genes in chronic AD when compared with acute AD, including genes involved in epidermal differentiation (and response to barrier stress (IL-20, *KRT16*, *KRT6B*, *S100A8 and S100A9),* antimicrobial and immunomodulatory chemokines (*CXCL1, CXCL6)*, and negative T cell regulation (*TNIP3, CLEC3A*) and Th2 cell differentiation (IL-19).
- FOXK1 may have a role as a negative immune regulator in chronic AD

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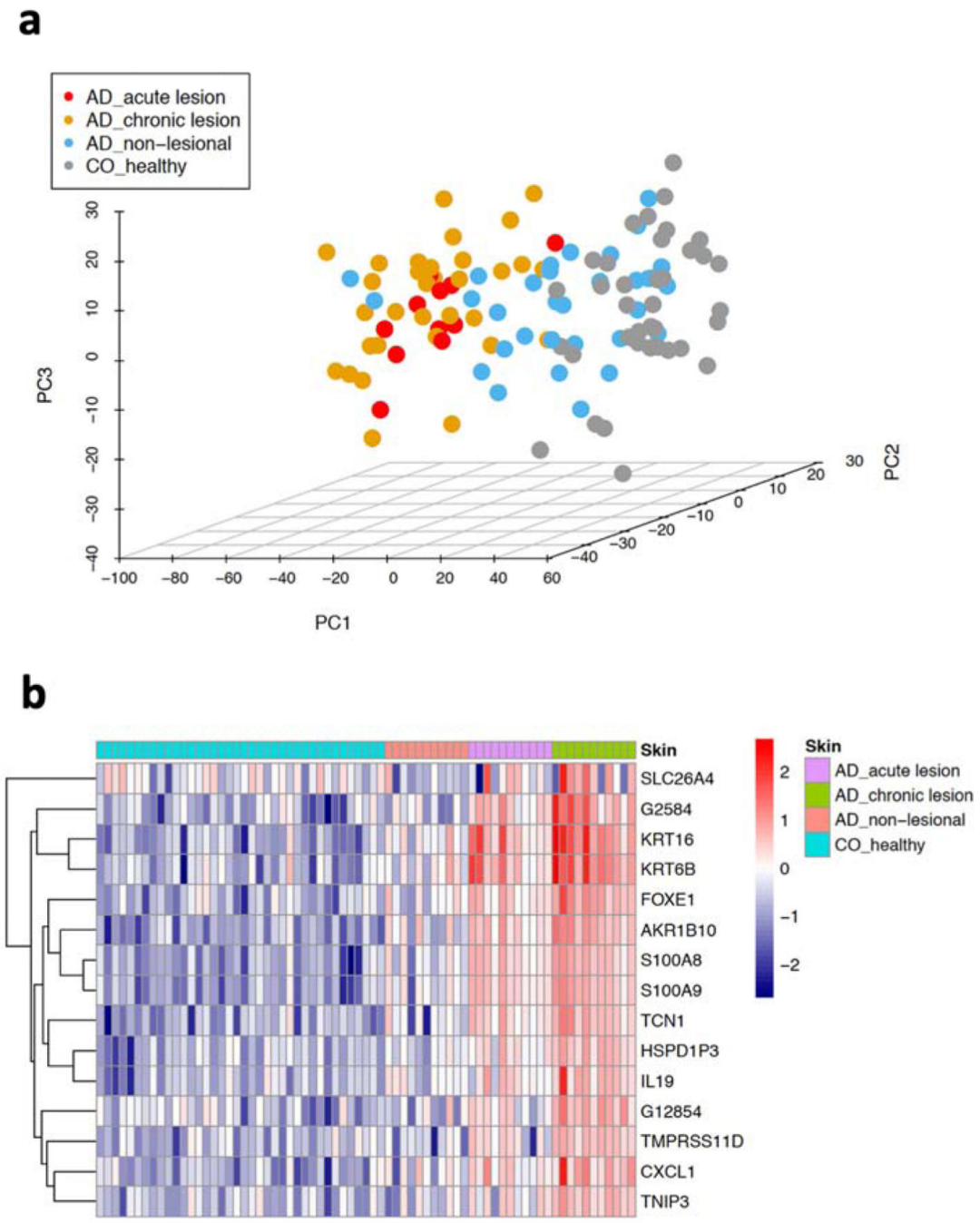

Figure 1. Transcriptomic landscape and progressive profiles in AD disease stages.

a) the top three principal components computed using the whole transcriptomic data; b) heatmap illustrating the expression profiles for genes with progressive pattern of up-regulation from non-lesional to acute to chronic AD.

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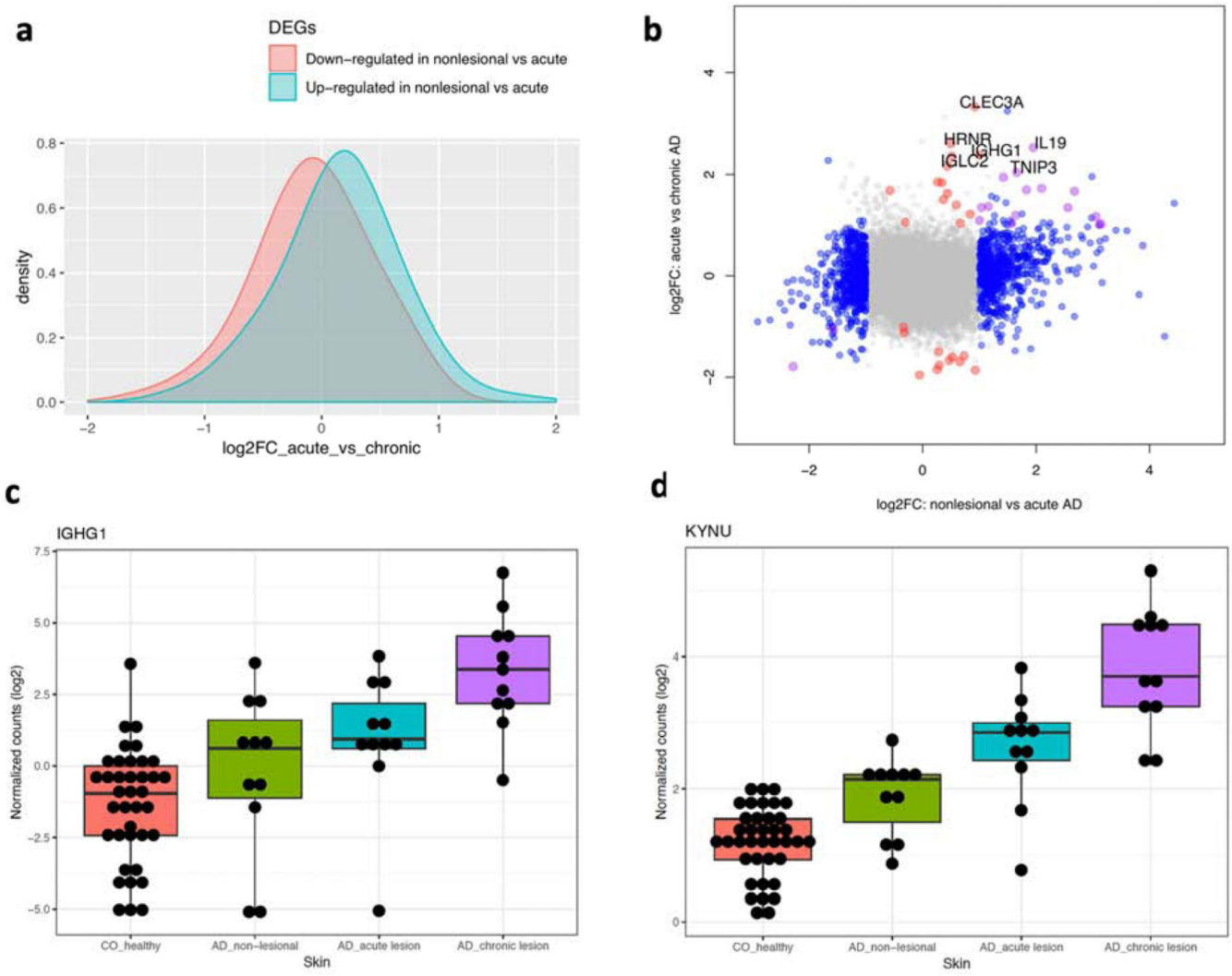

Figure 2. Molecular profiling for AD progression from acute to chronic.

a) effect size in the acute vs chronic AD comparison for genes dysregulated in non-lesional vs. acute AD; b) effect size comparison between non-lesional vs. acute AD (x-axis) against acute AD vs. chronic AD (y-axis). Genes are colored if they are dysregulated in the first (blue), second (red) or both comparisons (purple); c-d) boxplots to illustrate the expression profiles for 2 genes dysregulated in the acute vs. chronic comparison but not in the non-lesional vs. acute comparison.

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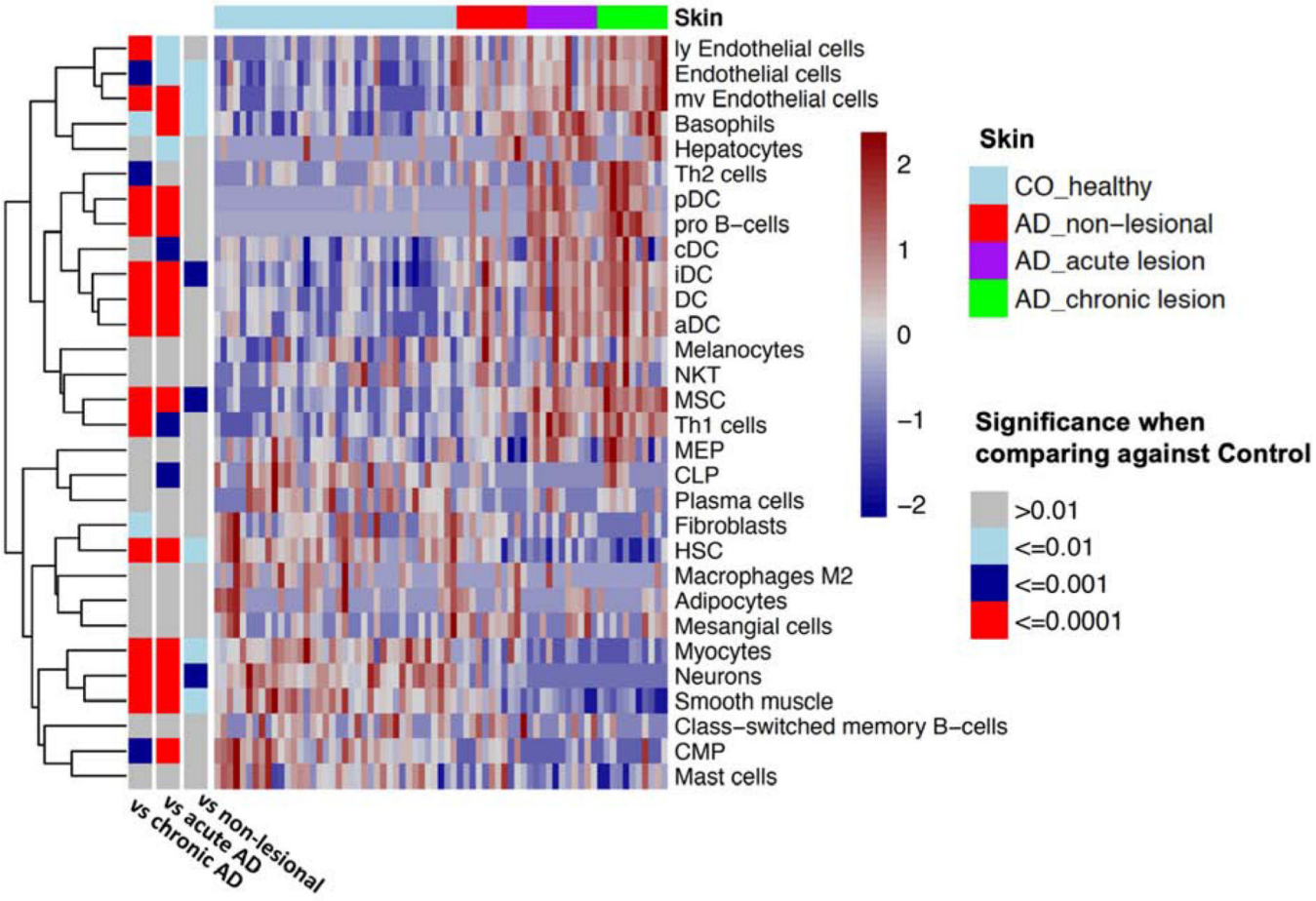

Figure 3. Heatmap to indicate the presence of cell type signature comparing control vs different AD disease stages.

The color in the heatmap correlates with the presence of the cell type specific signature. The bars on the left indicate the significance level for the difference in cell type signatures when comparing control with different AD skin types.

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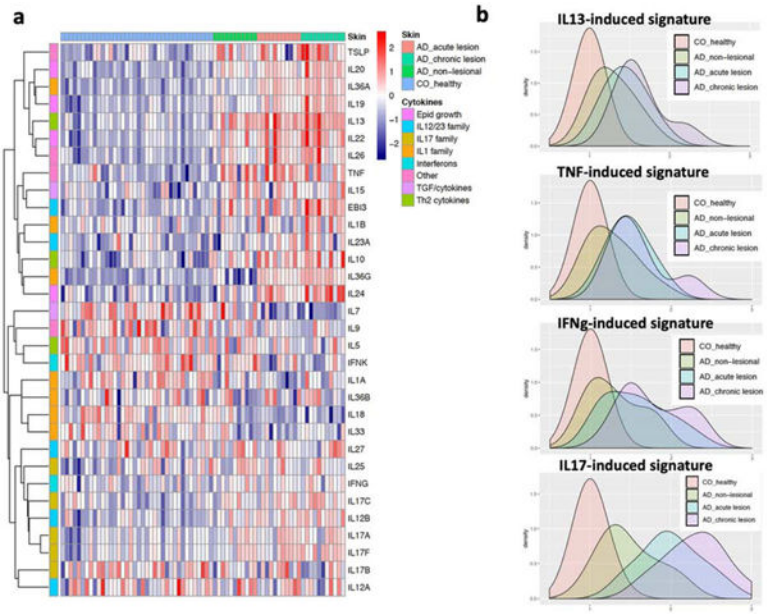

Figure 4. Cytokine expression and effect in AD.

a) heatmap shows the expression profiles for different cytokines in healthy skin, non-lesional, acute and chronic AD subtypes; b) distributions of the cytokine stimulated “burden” in healthy skin, non-lesional, acute and chronic stages of AD. The values are normalized referencing the control samples.

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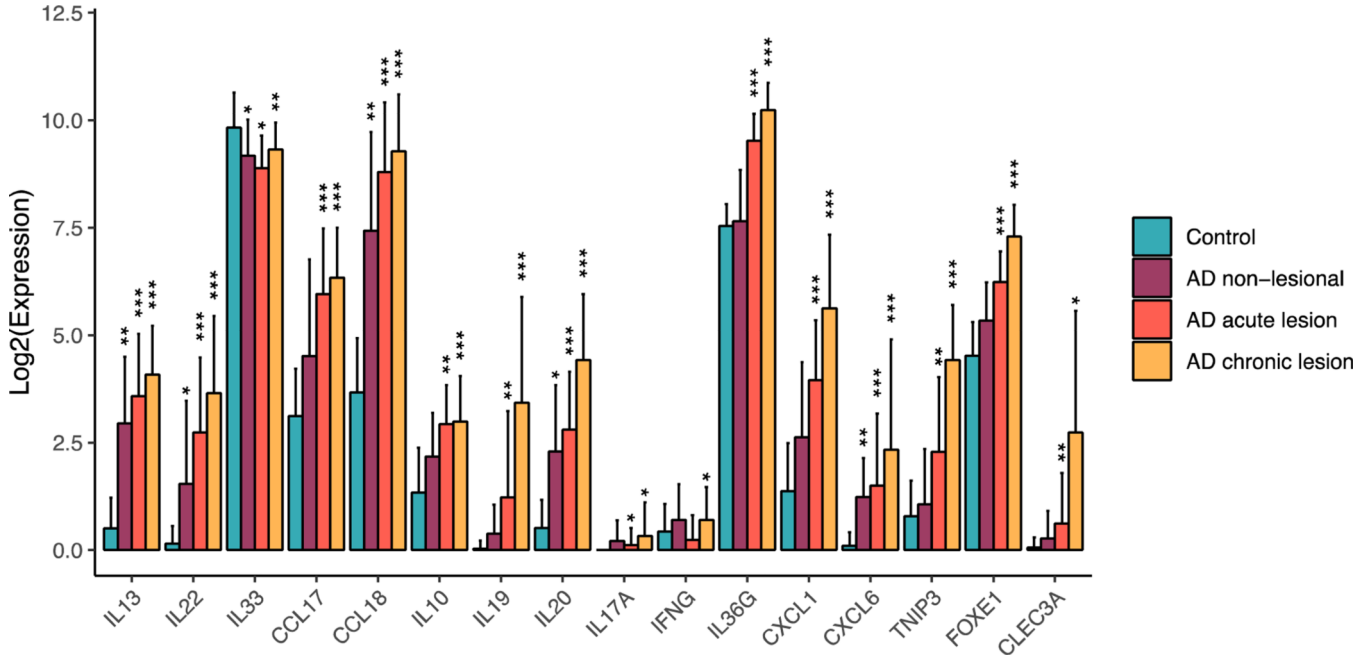

Figure 5. Changes in gene expression from control to different types of AD skin.
*$FDR \leq 0.1$; **$FDR \leq 0.01$; ***$FDR \leq 1 \times 10^{-5}$

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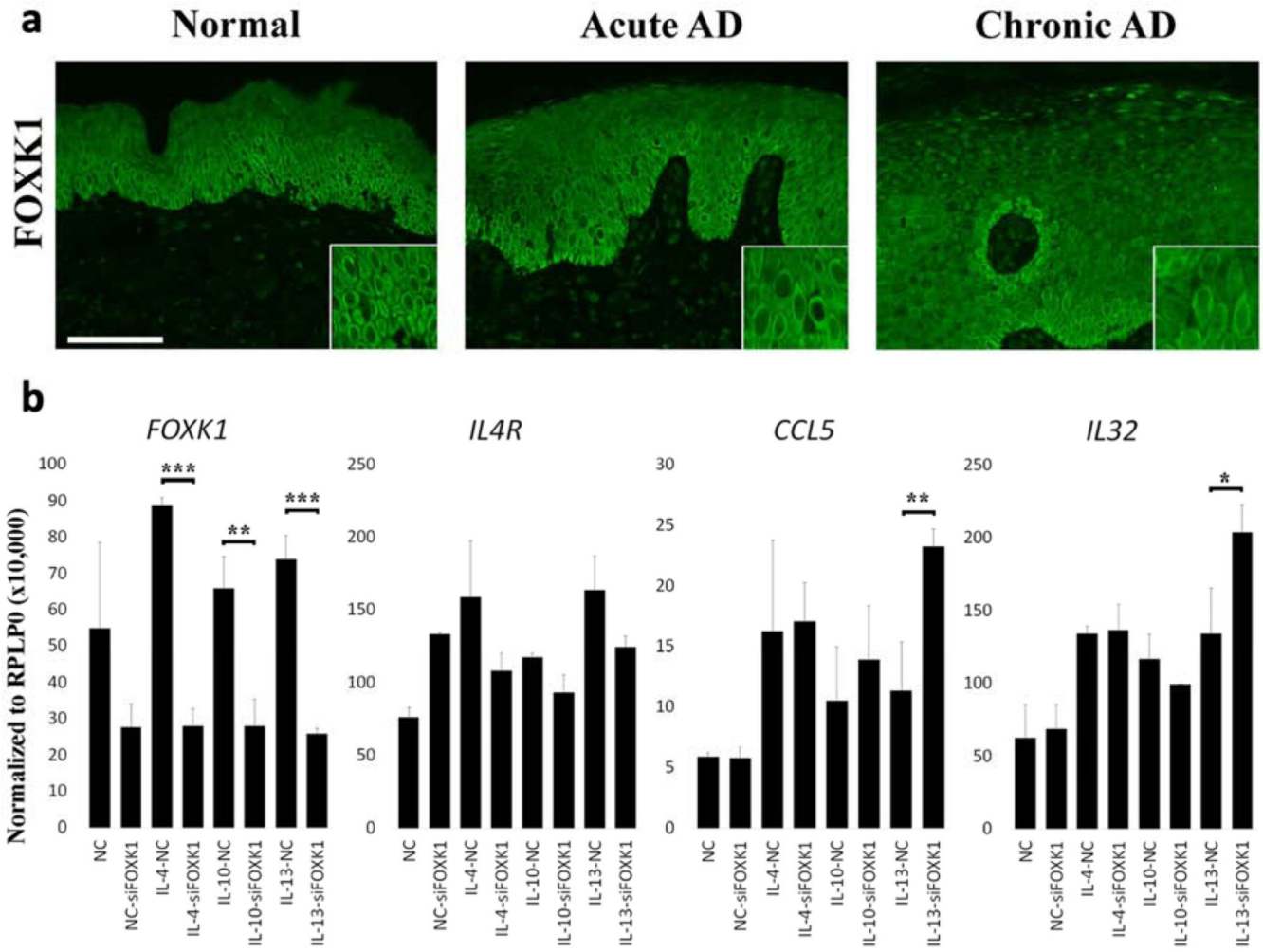

Figure 6. Expression of FOXK1 in AD and its modulating effect upon cytokine stimulation.

a) immunostaining for FOXK1 in normal, acute, and chronic AD lesional skin; b) RNA expression (from RT-PCR) of *FOXK1*, *IL4R*, *CCL5*, and *IL32* upon the indicated cytokine stimulation in keratinocytes under control (NC) or *FOXK1* knockdown (siFOXK1). *$p<0.05$; **$p<0.01$; ***$p<0.001$.