

IL-22-producing “T22” T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing T_H17 T cells

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Background: Psoriasis and atopic dermatitis (AD) are common inflammatory skin diseases. An upregulated T_H17/IL-23 pathway was demonstrated in psoriasis. Although potential involvement of T_H17 T cells in AD was suggested during acute disease, the role of these cells in chronic AD remains unclear. **Objective:** To examine differences in IL-23/T_H17 signal between these diseases and establish relative frequencies of T-cell subsets in AD.

Methods: Skin biopsies and peripheral blood were collected from patients with chronic AD (n = 12) and psoriasis (n = 13). Relative frequencies of CD4⁺ and CD8⁺ T-cell subsets within these 2 compartments were examined by intracellular cytokine staining and flow cytometry.

Results: In peripheral blood, no significant difference was found in percentages of different T-cell subsets between these diseases. In contrast, psoriatic skin had significantly increased frequencies of T_H1 and T_H17 T cells compared with AD, whereas T_H2 T cells were significantly elevated in AD. Distinct IL-22–producing CD4⁺ and CD8⁺ T-cell populations were significantly increased in AD skin compared with psoriasis. IL-22⁺CD8⁺ T-cell frequency correlated with AD disease severity.

Conclusion: Our data established that T cells could independently express IL-22 even with low expression levels of IL-17. This argues for a functional specialization of T cells such that “T17” and “T22” T-cells may drive different features of epidermal pathology in inflammatory skin diseases, including induction of antimicrobial peptides for “T17” T cells and

epidermal hyperplasia for “T22” T-cells. Given the clinical correlation with disease severity, further characterization of “T22” T cells is warranted, and may have future therapeutic implications. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Atopic dermatitis, psoriasis, T_H17, IL-17, IL-22, T22

Psoriasis and atopic dermatitis (AD) are common inflammatory skin diseases previously described as opposite poles of the T_H1/T_H2 paradigm. Recently, interleukin (IL)-17–producing T_H17 T cells have challenged this classic paradigm, and they are implicated in a growing number of inflammatory diseases. Our group and others have shown increased T_H17 T cells in psoriasis, and enhanced expression of IL-17 and IL-23 in lesional psoriatic skin.^{1,2} The IL-23/T_H17 pathway is thought to contribute to the pathogenesis of psoriasis and to the distinct features of the disease (ie, neutrophil chemotaxis and increased expression of key antimicrobial peptides).³ Recently, elegant evidence that IL-17–producing CD8⁺ T cells may also contribute to the pathogenesis of psoriasis was presented.⁴ Whereas the majority of the CD4⁺ T_H17 cells were observed in the psoriatic dermis, IL-17⁺CD8⁺ T cells were mostly in psoriatic epidermis.⁴

In murine systems, T_H17 T cells have also been recognized as the main producers of another cytokine, IL-22,^{5,6} although a distinct population of human CD4⁺ T cells that produce IL-22, without the cosynthesis of IL-17 or IFN- γ , was previously identified.³ In the skin, IL-22 mediates keratinocyte proliferation and epidermal hyperplasia by downmodulating terminal keratinocyte differentiation genes.^{3,7,8} Hence, IL-22 is thought to play a central role in inflammatory diseases with marked epidermal acanthosis, such as psoriasis.^{3,5,8-10}

Although the role of IL-17⁺ T cells is well defined in psoriasis, their role in AD is still unclear. A potential involvement of T_H17 T cells in AD has been suggested, at least during onset of the disease.¹¹ Toda et al¹¹ have demonstrated by immunohistochemistry that IL-17⁺ cells are increased in acute, but not chronic, AD lesions compared with uninvolved or normal skin. Koga et al¹² confirmed the immunohistochemistry results on acute AD skin (although IL-17 expression was not associated with a specific T-cell population), identified CD4⁺ T cells as the major producers of IL-17 in peripheral blood, and correlated the percentage of circulating T_H17 T cells with increased severity of the disease. In contrast, our group found decreased expression of IL-23, IL-17, and IL-17–induced innate defense molecules in chronic AD lesions compared with psoriasis that suggest a relative deficiency of the IL-23/T_H17 pathway in AD.¹³

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Abbreviations used

AD: Atopic dermatitis
AMP: Antimicrobial peptide
FACS: Fluorescence-activated cell sorting
SCORAD: Scoring Atopic Dermatitis
Tc: CD8 T cell

Although immunohistochemistry provides important evidence of IL-17 protein expression, the identification and measurement of T-cell subsets by this method may underestimate actual cell frequencies by virtue of T-cell cytokine secretion. Because the relative frequencies of different CD4⁺ and CD8⁺ T cell subsets in AD skin lesions are currently unknown, we sought to establish these relative frequencies by analyzing lymphocytes directly isolated from skin biopsies or peripheral blood of patients with chronic AD and psoriasis using intracellular cytokine staining and flow cytometry.

We confirmed that psoriasis was polarized toward a T_H1/T_H17 phenotype, whereas AD was polarized toward T_H2 with relatively deficient T_H17 activity. Interestingly, we found that AD skin had up-regulated expression of IL-22, associated with increased frequencies of distinct IL-22-producing CD4⁺ and CD8⁺ T cells. This upregulation of IL-22, independent from T_H17 cells, might explain the discordance between reactive epidermal hyperplasia and deficient IL-17-related innate defense molecules observed in chronic AD.

METHODS**Study design**

Peripheral blood and skin biopsies were collected from 12 patients with AD (age 23–48 years; median, 34 years) and 13 patients with psoriasis (age 28–60 years; median, 52 years) under a Rockefeller University institutional review board–approved protocol (Table I). Patients with moderate to severe psoriasis and >10% body surface area involvement, and patients with acute exacerbation of chronic AD (Scoring Atopic Dermatitis [SCORAD] between 21 and 72), were included in the study. A therapeutic washout period of 4 weeks for oral medication and 2 weeks for topical treatment was implemented before specimen collection.

Skin samples

Skin punch biopsies (5 mm diameter) were incubated at 4°C overnight in dispase (Invitrogen Life Technologies, Carlsbad, Calif). After the epidermis was peeled off, the dermis was cultured for 48 hours at 37°C in RPMI 1640 (Gibco-BRL Life Technologies) supplemented with 10% pooled human serum (Mediatech Inc, Manassas, Va), 0.1% gentamicin reagent solution (Gibco-BRL Life Technologies), and 1% 1 mol/L HEPES buffer (Sigma, St Louis, Mo). Single-cell suspensions were obtained from culture media, washed with PBS, and analyzed by intracellular cytokine staining and fluorescence-activated cell sorting (FACS).

Peripheral blood samples

PBMCs were obtained by gradient centrifugation with Ficoll-Paque Plus (Amersham Biosciences, Pittsburgh, Pa), collected at the interface, and washed with PBS before intracellular cytokine staining and FACS analysis.

FACS

For intracellular cytokine staining, cells were activated for 4 hours by using 25 ng/mL phorbol 12-myristate 13-acetate and 2 µg/mL ionomycin in the presence of 10 µg/mL brefeldin A (all Sigma Aldrich) at 37°C. Unactivated controls were treated with brefeldin A only. EDTA (2 mmol/L, Fisher

TABLE I. Profiles of patients with AD and psoriasis

AD	Age (y)	IgE	Eos (%)	SCORAD	Atopic background	Psoriasis	Age (y)	PASI
1	30	2000	2.10	56	Yes	1	28	29.2
2	34	1512	11.10	58	No	2	53	28.3
3	48	39	4.30	37	No	3	52	39.6
4	38	2000	0.10	72	No	4	58	31.2
5	26	ND	0.40	30	No	5	60	36.9
6	43	ND	0.70	52	No	6	54	34.8
7	29	220	0.30	29	Yes	7	35	23.4
8	42	461	1.30	54	No	8	45	19.3
9	23	152	0.70	59	Yes	9	41	16.2
10	34	ND	ND	37	Yes	10	56	19.2
11	36	ND	ND	23	Yes	11	59	21.1
12	24	1121	0.30	21	Yes	12	46	37.8
						13	35	33.4

Eos, Eosinophil count; PASI, Psoriasis Area and Severity Index; ND, not determined.

Scientific, Pittsburg, Pa) was added for 10 minutes at 37°C to stop activation. Cells were then incubated in aqua marina live/dead dye (Invitrogen) for 30 minutes for dead cell discrimination, then fixed with 4% paraformaldehyde (BD Biosciences, San Jose, Calif) for 20 minutes. The cells were washed, blocked in 1:100 mouse serum (BD Biosciences), permeabilized in FACSPERM (BD Biosciences), incubated for 30 minutes with fluorochrome conjugated mAbs to cell surface molecules and intracellular cytokines, washed, and collected. Cells were stained with antibodies listed in this article's Table E1 in the Online Repository at www.jacionline.org. Samples were acquired by using an LSR-II flow cytometer (BD Biosciences) and analyzed with FlowJo software (Treestar, Ashland, Ore). All incubation steps were done on ice. Appropriate isotype controls were used.

Real-time PCR

RNA was extracted from human skin by using the RNeasy Mini Kit (Qiagen). RT-PCR was performed by using EZ PCR core reagents, primers, and probes (Applied Biosystems) as per the manufacturer's recommendations. Sequences of primers and probes used were as follows: IFN-γ, Hs99999041_m1; IL-13, Hs00174379_m1; IL-17, Hs00174383_m1; and IL-22, Hs00220924_m1. The data were analyzed by software provided with the Applied Biosystems PRISM 7700 (Sequence Detection Systems, v 1.7) and normalized to the human acidic ribosomal protein (hARP) housekeeping gene.

Immunofluorescence

Frozen tissue sections from patients with AD (n = 5) were fixed with acetone and blocked in 10% normal goat serum (Vector Laboratories, Burlingame, Calif) for 30 minutes. The primary antibody, anti-CD8–fluorescein isothiocyanate (BD Biosciences), was incubated overnight at 4°C and amplified with the secondary antibody, goat anti-fluorescein IgG–Alexa 488 (Invitrogen), for 30 minutes. Images were acquired by using the appropriate filters of a Zeiss Axioplan 2 widefield fluorescence microscope with a Plan Neofluar 10 x 0.30 N.A. lens and a Hamamatsu Orca ER-cooled CCD camera, controlled by METAVUE software (MDS Analytical Technologies, Downingtown, Pa).

Statistical analysis

The 2-tailed Student *t* test was used to compare values obtained from patients with AD and psoriasis. The *P* values were designated as **P* < .05, ***P* < .01, and ****P* < .005. Linear regression was used to correlate T-cell frequencies and disease activity.

RESULTS

Adult patients were studied with active lesions of chronic AD (n = 12) and psoriasis (n = 13) and clinical and demographic characteristics described in Table I.

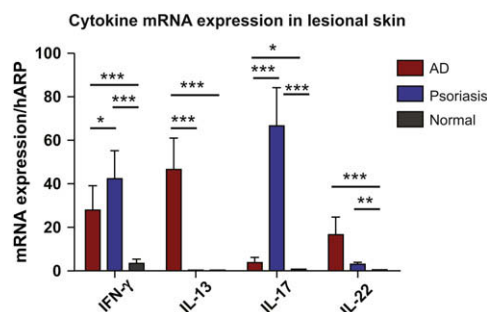


FIG 1. The cytokine microenvironment in AD and psoriasis skin lesions. Quantitative RT-PCR analysis of cytokine mRNA expression normalized to hARP in skin biopsies of lesional AD, psoriasis, and normal skin. T_H1/T_H17 (IFN- γ /IL-17) polarization was observed in psoriasis, T_H2 (IL-13) polarization in AD, with upregulation of IL-22 expression in both diseases. * $P < .05$; ** $P < .01$; *** $P < .005$.

IL-22 expression is significantly upregulated in AD compared with psoriasis skin lesions

To examine the cytokine microenvironment in AD and psoriasis skin, we quantified IFN- γ , IL-13, IL-17, and IL-22 mRNA expression in lesional skin compared with normal skin by real-time RT-PCR. As expected, T_H1 -associated IFN- γ mRNA was significantly upregulated in psoriasis lesions compared with AD ($P < .05$) and normal skin ($P < .005$) (psoriasis, 42.37 ± 12.83 ; AD, 27.94 ± 11.22 ; normal skin, 3.46 ± 1.91), whereas T_H2 cytokine IL-13 was highly expressed in AD lesions and absent in psoriatic and normal skin (psoriasis, 0.00 ± 0.00 ; AD, 46.65 ± 14.37 ; normal skin, 0.00 ± 0.00 ; $P < .005$). The expression of T_H17 -defining cytokine, IL-17, was significantly upregulated in psoriasis lesions compared with AD and normal skin (psoriasis, 66.31 ± 17.56 ; AD, 3.75 ± 2.43 ; normal skin, 0.00 ± 0.00 ; $P < .005$). As expected, IL-22 was significantly upregulated in psoriasis compared with normal skin ($P < .01$). Unexpectedly, IL-22 was also upregulated in AD lesional skin compared with normal skin ($P < .005$), with a higher expression in AD than in psoriatic lesions (psoriasis, 3.02 ± 0.91 ; AD, 16.62 ± 8.10 ; normal skin, 0.00 ± 0.00 ; Fig 1).

Similar frequencies of circulating T-cell subsets in AD and psoriasis

To determine relative frequencies of different T-cell subsets that may contribute to cytokine polarization in skin lesions, we analyzed T_H ($CD3^+CD4^+$) and T-cytotoxic ($CD3^+CD8^+$) cells in peripheral blood from patients with AD and psoriasis by intracellular cytokine staining and flow cytometry (Fig 2, A). We defined IFN- γ -producing, IL-4-producing, and IL-17-producing T_H cells as T_H1 , T_H2 , and T_H17 cells, respectively. In the same manner, IFN- γ -producing, IL-4-producing, and IL-17-producing $CD8^+$ T cells were defined as Tc1, Tc2, and Tc17 cells, respectively.

We found that the percentages of peripheral blood T_H1 , T_H2 , or T_H17 T cells did not differ significantly between the patients with AD and psoriasis ($CD4^+/T_H1$: psoriasis, $8.24\% \pm 1.11\%$, vs AD, $9.15\% \pm 1.34\%$; T_H2 : psoriasis, $1.34\% \pm 0.20\%$, vs AD, $1.18\% \pm 0.15\%$; T_H17 : psoriasis, $0.39\% \pm 0.08\%$, vs AD, $0.50\% \pm 0.09\%$; Fig 2, B). Similarly, the percentages of Tc1, Tc2, and Tc17 T cells in peripheral blood did not differ significantly between the 2 diseases ($CD8^+/Tc1$: psoriasis, $26.54\% \pm 5.24\%$, vs AD, $24.17\% \pm 3.82\%$; Tc2: psoriasis, $2.07\% \pm 0.832\%$, vs

AD, $0.55\% \pm 0.09\%$; Tc17: psoriasis, $0.11\% \pm 0.02\%$, vs AD, $0.06\% \pm 0.02\%$). The frequencies of IL-22-producing $CD4^+$ and $CD8^+$ T cells in peripheral blood also did not differ significantly between patients with psoriasis and AD ($CD4^+IL-22^+$: psoriasis, $0.98\% \pm 0.12\%$, vs AD, $1.45\% \pm 0.26\%$; $CD8^+IL-22^+$: psoriasis, $0.44\% \pm 0.13\%$, vs AD, $0.73\% \pm 0.27\%$; Fig 2, B).

Discordant frequencies of T-cell subsets in skin lesions of AD and psoriasis

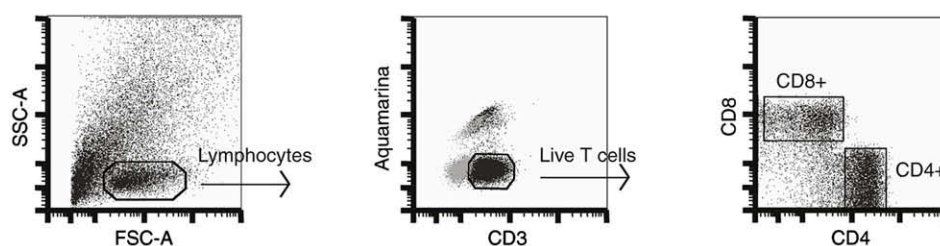
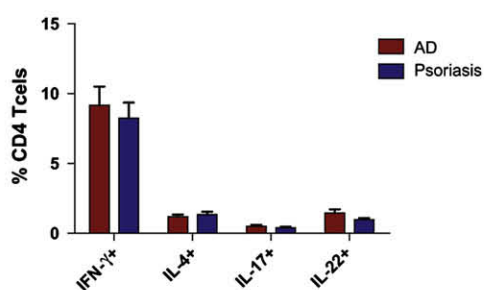
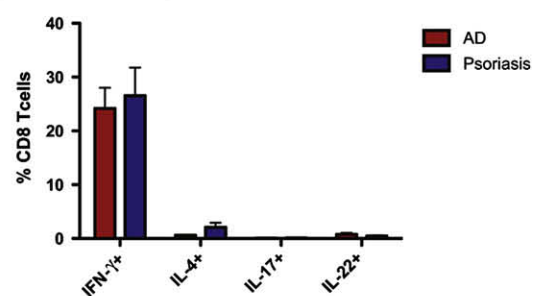
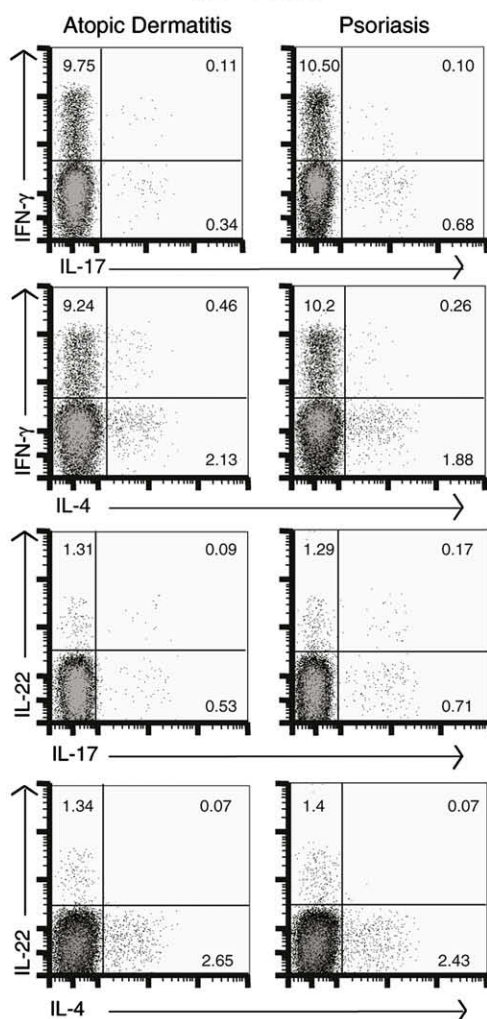
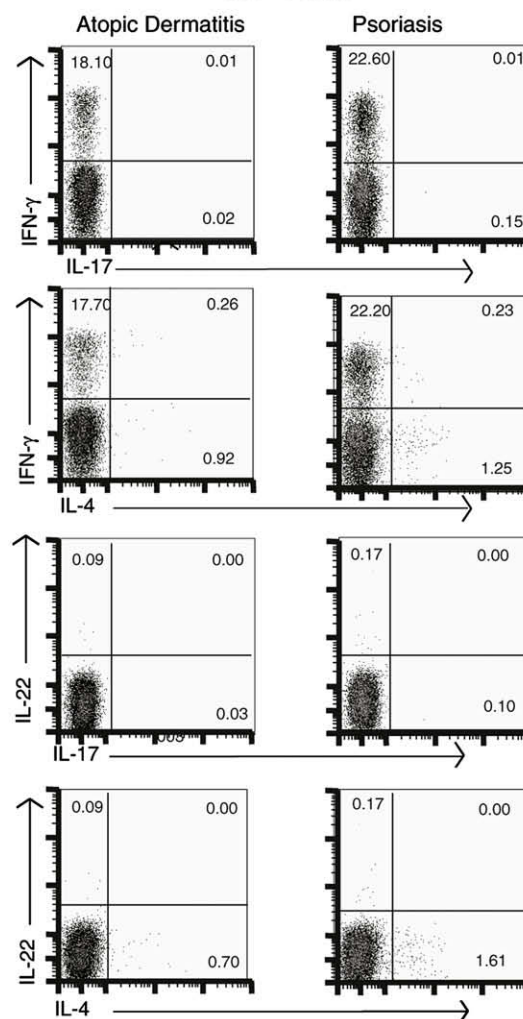
We obtained direct T-cell isolates from skin biopsies and determined their cytokine synthesis capability by using the same FACS gating strategy as in peripheral blood. We confirmed that the frequency of IFN- γ -producing T_H1 T cells was significantly increased in psoriasis compared with AD (psoriasis, $13.90\% \pm 1.31\%$; AD, $8.27\% \pm 1.47\%$; $P < .01$), whereas the frequency of IL-4-producing T_H2 T cells was significantly higher in AD compared with psoriasis (psoriasis, $1.53\% \pm 0.30\%$; AD, $5.04\% \pm 0.95\%$; $P < .005$; Fig 3, A). We also observed a lower frequency of IL-17-producing T_H17 T cells in AD lesions compared with psoriasis (psoriasis, $7.82\% \pm 1.03\%$; AD, $4.23\% \pm 0.75\%$; $P < .05$). However, despite the relative T_H17 deficiency in AD compared with psoriasis, the mean percentages of IL-22-producing $CD4^+$ T cells were similar in both diseases (psoriasis, $8.01\% \pm 0.82\%$; AD, $10.86\% \pm 3.22\%$; Fig 3, A). Although the percentages of IL-22 $^+CD4^+$ T cells were similar between AD and psoriasis, the majority of the IL-22 production in AD was not derived from IL-17 $^+T_H17$ cells, with only a small proportion of cells co-synthesizing both IL-17 and IL-22 (Fig 3, A, FACS plots, third panel from top). Although only a small proportion of IL-4 $^+T_H2$ T cells were shown to produce IL-22 in both diseases, the frequency of IL-22-producing T_H2 T cells in AD was significantly higher than in psoriasis (psoriasis, $0.12\% \pm 0.02\%$; AD, $0.72\% \pm 0.24\%$; $P < .005$; Fig 3, A, FACS plots, fourth panel from top).

$CD8^+$ T cells accounted for approximately 20% of total T cells in both AD and psoriasis skin biopsies (see this article's Fig E1 in the Online Repository at www.jacionline.org). In psoriasis, majority of the $CD8^+$ T cells were IFN- γ -producing Tc1 cells (psoriasis, $41.62\% \pm 2.90\%$; Fig 3, B). Tc1 T cells were also found in AD, although at a significantly lower frequency than in psoriasis (AD, $28.86\% \pm 3.37\%$; $P < .01$). There were very few IL-4 (Tc2) or IL-17 (Tc17)-producing $CD8^+$ T cells in both AD and psoriasis lesions. Notably, IL-22-producing $CD8^+$ T cells were significantly increased in AD lesions compared with psoriasis (psoriasis, $3.50\% \pm 0.42\%$; AD, $20.56\% \pm 6.56\%$; $P < .05$; Fig 3, B), and a majority of these cells did not co-synthesize IL-17 or IL-4 (Fig 3, B, FACS plots, third and fourth panels from top).

Unique IL-22-producing $CD4$ and $CD8$ T cells are increased in AD

To characterize further the IL-22-producing T cells in AD, we localized IL-22 production within the different $CD4^+$ and $CD8^+$ T-cell subsets in AD and psoriasis skin. FACS gates were first set on all $CD4^+$ or $CD8^+$ T cells synthesizing IL-22, and then co-synthesis of IFN- γ , IL-4, and IL-17 was determined within these IL-22 $^+$ T cells (Fig 4, A-C).

In psoriasis, IL-22 production within $CD4^+$ T-cell subsets was observed in T_H1 ($10.43\% \pm 1.3\%$) and T_H17 ($39.17\% \pm 3.4\%$) cells. However, the largest subset of IL-22-producing $CD4^+$ T cells ($49.90\% \pm 3.01\%$) did not co-secrete IFN- γ , IL-4, or IL-17 (Fig 4, B).

A. Gating strategy**B. Peripheral blood**Cytokine producing CD4⁺ T cells in peripheral bloodCytokine producing CD8⁺ T cells in peripheral blood**CD4⁺ T cells****CD8⁺ T cells**

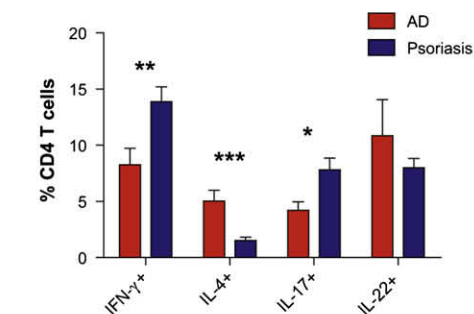
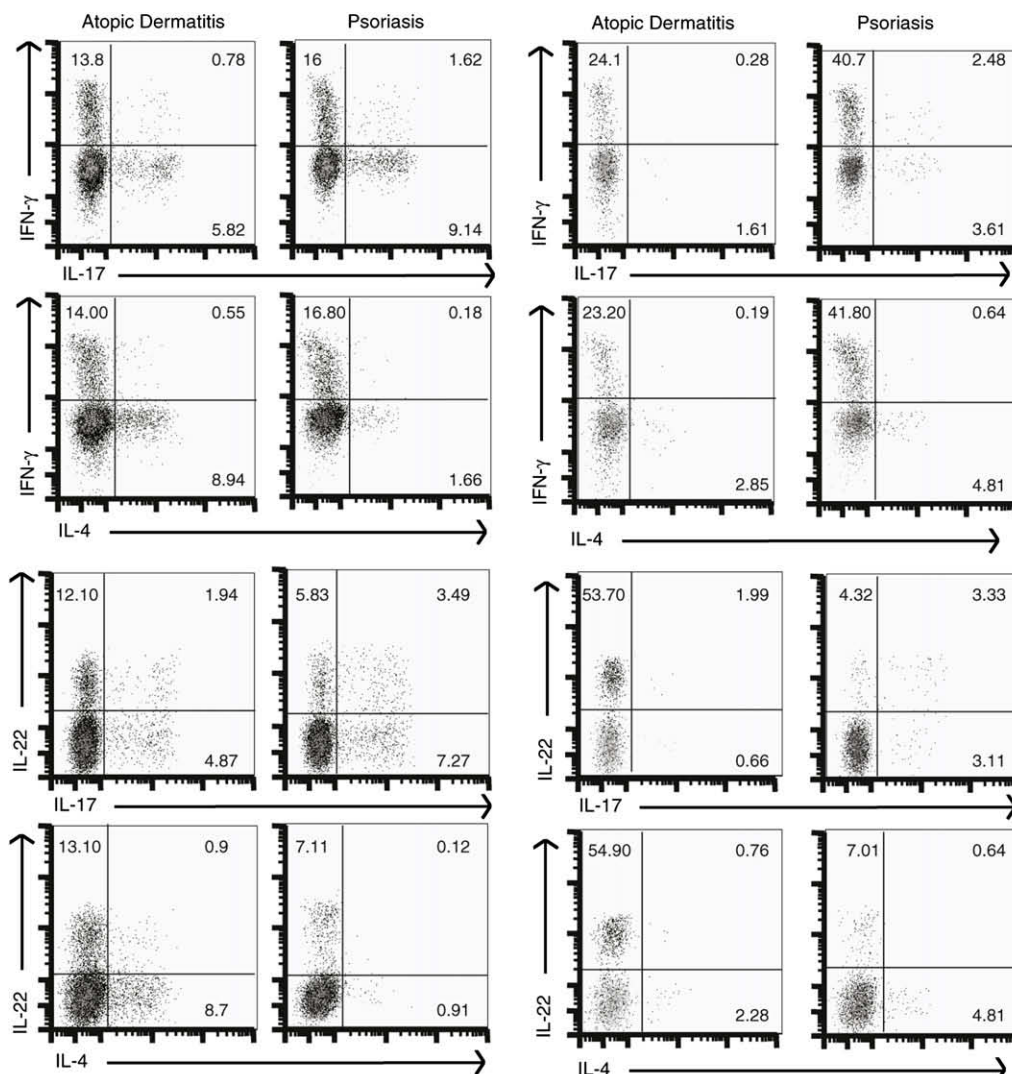
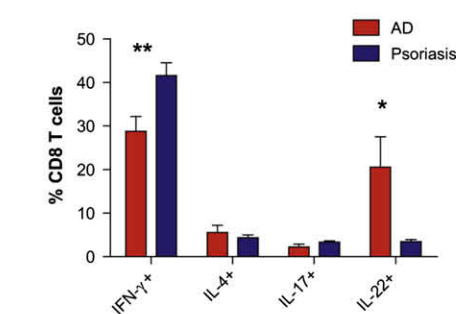
A. CD4⁺ T cells in lesional skin**B. CD8⁺ T cells in lesional skin**

FIG 3. AD and psoriasis lesions have distinct T-cell profiles. **A**, Graph of CD4⁺ T-cell subsets in AD and psoriasis lesions showing T_H1/T_H17 (IFN-γ/IL-17) polarization in psoriasis, T_H2 (IL-4) in AD, and similar IL-22⁺CD4⁺ frequencies in both. Representative FACS plots follow. **B**, Graph of skin CD8⁺ T-cell subsets showing Tc1 (IFN-γ) polarity in psoriasis and IL-22⁺CD8⁺ polarity in AD. Representative FACS plots follow. **P* < .05; ***P* < .01; ****P* < .005.

FIG 2. Circulating CD3⁺ T-cell profiles are similar in AD and psoriasis. **A**, FACS gating used in the analysis of peripheral blood. **B**, Frequencies of circulating cytokine-producing CD4⁺ and CD8⁺ T cells did not differ between AD and psoriasis. *Graphs* summarize cytokine-producing CD4⁺ and CD8⁺ T-cell subsets in AD and psoriasis blood. Representative FACS plots are shown. *SSC-A*, side scatter; *FSC-A*, forward scatter.

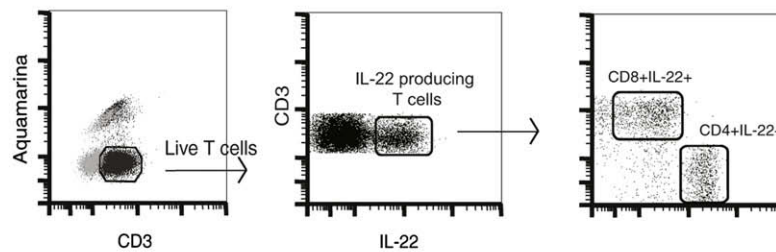
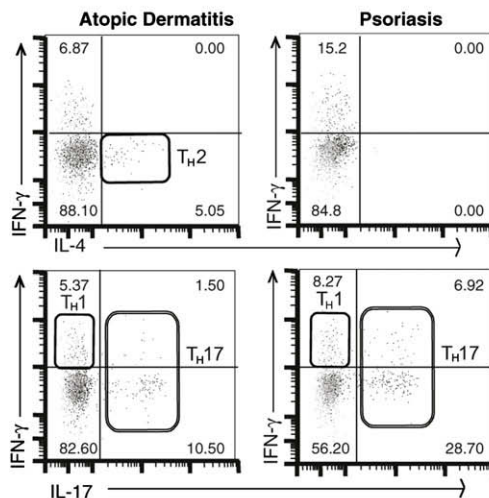
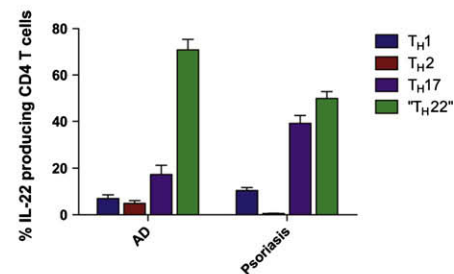
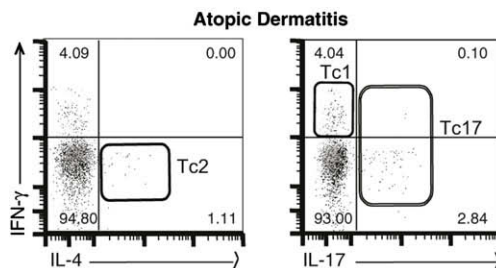
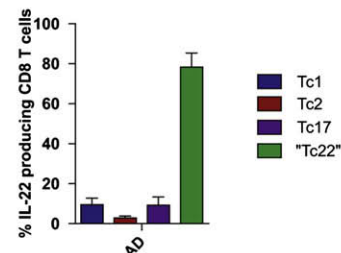
A. Gating strategy**B. CD4+IL-22+ T cells****Localization of IL-22 production by CD4+ T cells****C. CD8+IL-22+ T cells****Localization of IL-22 production by CD8+ T cells**

FIG 4. Unique IL-22-producing CD4⁺ and CD8⁺ subsets are abundant in AD lesions. **A**, FACS gating used in the analysis of IL-22-producing T cells. **B**, Majority of IL-22⁺ CD4⁺ T cells in AD are not T_H1, T_H2, or T_H17 cells. Psoriasis IL-22 is produced by T_H1, T_H17, and "T_H22" cells. Representative FACS plots shown. **C**, Majority of IL-22-producing CD8⁺ T cells in AD are "Tc22" cells.

In AD lesions, IL-22 production was noted within T_H1 (6.96% $\pm$ 1.64%), T_H2 (4.90% $\pm$ 1.17%), and T_H17 (17.31% $\pm$ 4.00%) cell subsets. However, the overwhelming majority of IL-22-producing CD4⁺ T cells in AD lesions (70.83% $\pm$ 4.54%) were not T_H1, T_H2, or T_H17 cells, and may represent a unique subset of IL-22-producing T_H cells (Fig 4, B).

The analysis of IL-22-producing CD8⁺ T-cell subsets in AD lesions showed that 9.55% $\pm$ 3.23% were Tc1 cells, 2.85% $\pm$ 1.00% were Tc2 cells, and 9.28% $\pm$ 4.10% were Tc17 cells. Again, the majority of IL-22-producing CD8⁺ T cells in AD (78.32% $\pm$ 7.05%) were not Tc1, Tc2, or Tc17 cells (Fig 4, C).

Frequency of IL-22-producing CD8⁺ T cells correlates with AD disease severity

Numerous CD8⁺ T cells were observed in the dermis of AD lesions by direct immunofluorescence (Fig 5, A). To determine whether the frequency of T-cell subsets correlated with clinical severity, we performed linear regression analysis. We found that the frequency of CD8⁺IL-22⁺ T cells positively correlated with the AD clinical severity index or SCORAD ($r = 0.78$; $P < .05$; Fig 5, B). Although a similar trend was noted for CD4⁺IL-22⁺ T cells, it did not reach statistical significance ($r = 0.39$; $P < .22$; Fig 5, C). No correlation was observed

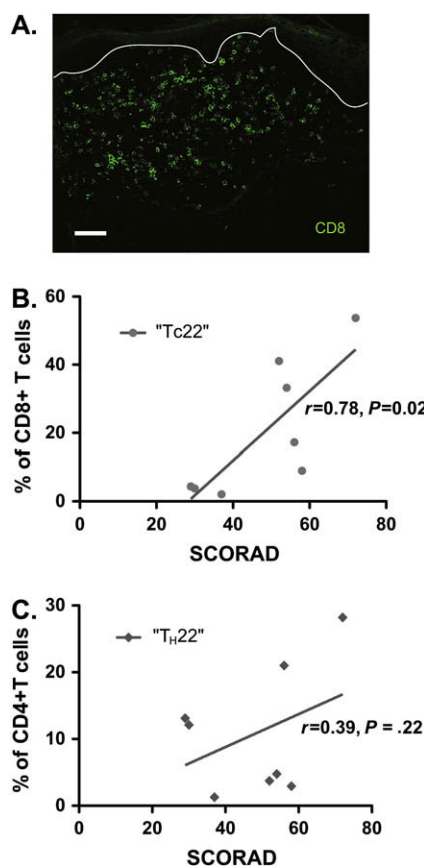


FIG 5. IL-22⁺ CD8⁺ T-cell frequency correlates with disease severity. **A**, Immunofluorescence showing abundant CD8⁺ T cells in AD dermis. Bar, 100 μ m. **B**, Linear regression showing positive correlation between IL-22-producing CD8⁺ T cells ("Tc22") and AD clinical severity (SCORAD; $r = 0.78$; $P < .05$). **C**, Linear regression of IL-22-producing CD4⁺ T cells ("TH22") showing positive trend with disease severity but not statistically significant.

between the frequencies of the other T-cell subsets and AD clinical severity (data not shown).

DISCUSSION

In this report, we explored the phenotype and cytokine production of different T-cell subsets in patients with chronic AD compared with psoriasis. Our findings expand current knowledge on the presence of T_H17 T cells and shed new light on a unique subset of IL-22-producing T cells in blood (Fig 2) and skin (Figs 3 and 4) compartments of patients with chronic AD.

We found a significantly decreased frequency of CD4⁺ T_H17 T cells in AD lesional skin compared with psoriasis, whereas comparable numbers of these cells were obtained in peripheral blood. However, no significant difference was observed in Tc17 cells in skin lesions of AD and psoriasis. We previously reported that, compared with psoriasis, there was little expression of IL-17 and IL-23 mRNAs in AD lesions.¹³ In the current study, we found a 2-fold difference in T_H17 T-cell frequencies between AD and psoriatic skin lesions and a 20-fold difference in IL-17 mRNA expression. The discrepancy between IL-17/IL-23 mRNA production and T_H17 T-cell frequencies could potentially mean that although T_H17 T cells exist in AD lesions, in contrast with psoriasis, they are not activated. Another possibility is that increased T_H2 cytokines in AD may directly inhibit IL-17 production.¹⁴⁻¹⁶

Evidence has been recently provided for an inhibiting effect of IL-4/IL-13 T_H2 cytokines on IL-17-induced antimicrobial protein secretion.¹⁷

Ultimately, several factors might collectively contribute to the reduction of T_H17/IL-17 in AD skin: (1) reduced recruitment of T_H17 T cells to AD skin because of either a lack of T_H17-attracting chemokines (eg, chemokine CC motif ligand 20 [CCL20] that is expressed at lower levels in AD skin), or a lack of T_H17-polarizing dendritic cells;¹⁸ (2) failure to activate existing T_H17 T cells, resulting in markedly decreased production of IL-17; and (3) active suppression of IL-17 production by antagonistic cytokines.^{5,14}

Increasing evidence suggests that IL-17 is a master regulator of antimicrobial peptides (AMPs) in keratinocytes, playing a central role in host defense against microorganisms at the surface barrier.^{17,19} Decreased IL-17 expression in chronic AD skin has been correlated to reduced expression of key AMPs, potentially accounting for the propensity to skin infections in this disease.^{13,17}

Nevertheless, the attenuated IL-17/AMP axis in chronic AD lesions does not explain the retained epidermal hyperplasia in this disease that bears histologic resemblance to psoriasis.^{18,19} A possible explanation for the hyperproliferative and acanthotic epidermis, as well as for disturbed terminal differentiation in AD, might be provided by IL-22. Surprisingly, despite the low frequency of T_H17 T cells, a significantly increased expression of IL-22 mRNA and upregulated production of IL-22 from CD4⁺ and CD8⁺ T cells was observed in lesional AD skin compared with psoriasis. Although IL-22 has some ability to induce antimicrobial peptides like S100A7, it has much stronger effects in regulating hyperplasia and differentiation in keratinocytes.^{3,5-7} In reconstructed epidermis, IL-22 induces marked acanthosis and hypogranulosis and suppression of terminal differentiation.⁸ *In vivo* overexpression of IL-22 induces marked hyperplasia and inflammation in murine skin, bearing similarities to both psoriatic and AD skin.⁹ Moreover, this cytokine downmodulates and inhibits genes involved in terminal differentiation of keratinocytes.^{3,7} Among these IL-22-regulated genes are several proteins recently implicated in the epidermal barrier defect in AD, including filaggrin, loricrin, and involucrin.²⁰⁻²⁵

Our data show that the majority of IL-22-producing T cells do not cosynthesize IFN- γ , IL-4, or IL-17. Although the CD4⁺ IL-22⁺ population is evident in both diseases, a significant proportion of IL-22 production is still derived from T_H17 T cells in psoriasis. In AD, a very large population of both CD4⁺ and CD8⁺ cells that uniquely synthesize IL-22 was appreciated, accounting for approximately 70% of the IL-22 production, with low frequencies of T_H1, T_H2, and T_H17 T cells that coproduce IL-22.

Although IL-22 production was mostly described in IL-17-producing T cells, a distinct IL-22-producing T-cell population has been previously detected in both mice⁵ and human beings.^{3,17} Our data establish that T cells could independently express IL-22 even with low expression levels of IL-17. This argues for a functional specialization of T cells such that "T17" and "T22" T cells may drive different features of epidermal pathology in inflammatory skin diseases, including induction of AMPs and proinflammatory chemokines by "T17" T cells, and keratinocyte proliferation and modulation of terminal differentiation by "T22" T cells (Fig 6). However, given the inhibitory effect of IL-4 on IL-17 production^{5,14} and on IL-17-induced AMPs,¹⁷ independent regulation of IL-17 and IL-22 production within a single T-cell population cannot be entirely excluded. Therefore, future experiments must determine whether unique IL-17 and

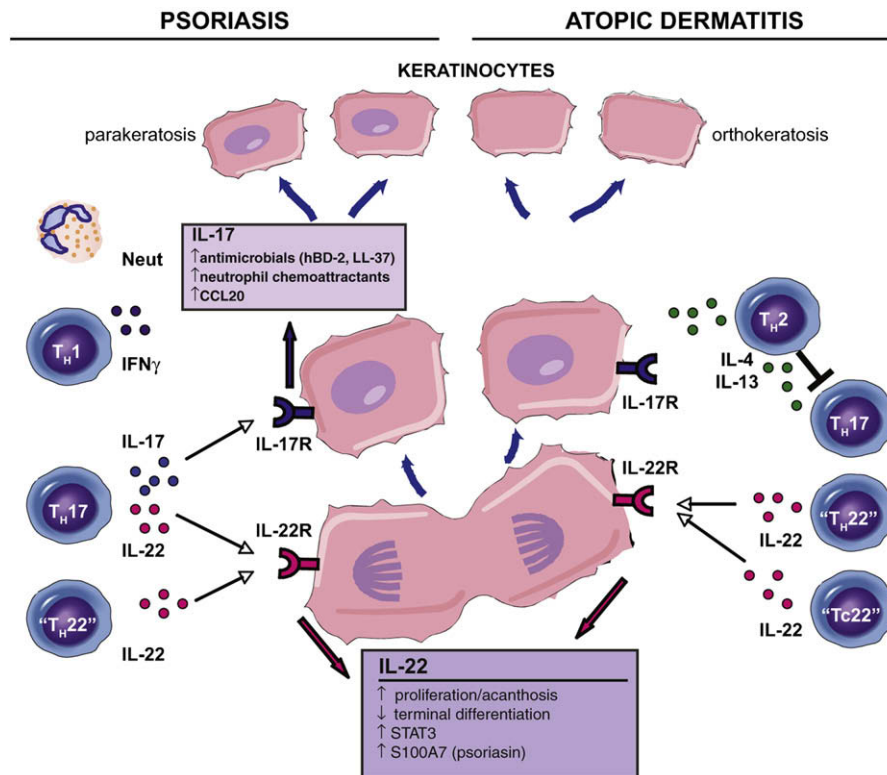


FIG 6. Model of major T cells and their cytokines in chronic skin lesions of psoriasis vs AD. In psoriasis, T_H1 cells produce $IFN-\gamma$, T_H17 cells produce both IL-17 and IL-22 cytokines, and “ T_H22 ” cells produce IL-22 but not IL-17. Upregulated IL-17 axis in psoriasis results in increased production of antimicrobial proteins, neutrophil chemoattractants, and CCL20 chemokine, whereas upregulated IL-22 causes epidermal acanthosis. In AD, the T_H2 cytokines IL-4 and IL-13 potentially inhibit T_H17 T-cell activity, resulting in reduced IL-17 effects. Both “ T_H22 ” and “ $Tc22$ ” T cells in AD are responsible for significantly increased IL-22 levels. The up-regulated IL-22 in chronic AD skin may explain the marked acanthosis that is histologically similar to psoriasis, and the defective terminal differentiation seen in this disease. *STAT*, Signal transducer and activator of transcription; *hBD-2*, human B-defensin 2; *LL-37*, cathelicidin; *IL-17R*, interleukin 17 receptor; *IL-22R*, interleukin 22 receptor.

IL-22-producing memory T-cell populations differentiate from naive T-cells, as opposed to the independent functional modulation of these cytokines within a common T-cell population.

Of particular interest is the $CD8^+IL-22^+$ T-cell population, which, although highly increased in AD skin, was negligible in psoriasis, precluding further analysis. Interestingly, we found a strong correlation between the frequency of $CD8^+IL-22^+$ “ $Tc22$ ” T cells and the AD disease severity index (Fig 5). This important observation expands a recently reported correlation between lesional $CD8^+$ T-cell frequencies with AD disease severity.²⁶ A critical role for $CD8^+$ T cells in AD was recently indicated in a mouse model that showed that these were the predominant cells responsible for development of AD skin pathology and inflammation.²⁷

In summary, our findings suggest that novel subsets of “ T_H22 ” and “ $Tc22$ ” T cells contribute to the increased IL-22 expression in chronic AD skin (Fig 6). Our results support a $T_H2/Tc22$ immune polarization in chronic AD, compared with the deviation toward a T_H1/T_H17 phenotype in psoriasis (Fig 6). The increased IL-22/“ $Tc22$ ” signal, despite a relative deficiency of $T_H17/IL-23$ pathway in AD, suggests an immune-driven hyperplasia by IL-22, independent of IL-17. Further support to this hypothesis stems from the small frequency of $IL-22^+$ T_H17 cells in AD.

Further elucidation of the role of these novel “ $Tc22$ ” T cells in both chronic and acute AD, as well as the interplay between these

cells and the $T_H17/IL-23$ pathway to produce the AD phenotype, is warranted. Our data, supported by the clinical correlation with disease severity, potentially suggest targeting “ $Tc22$ ” T cells in AD as a promising future therapeutic that may reverse both epidermal hyperplasia and the disturbed terminal differentiation pattern in this disease. Given the number of emerging biological therapies that directly target IL-22 or the $T_H17/IL-23$ pathway, these investigations become even more critical.

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Clinical implications: Increased IL-22 expression and IL-22-producing T cells in AD correlate with disease severity and may prove to be a future therapeutic target.

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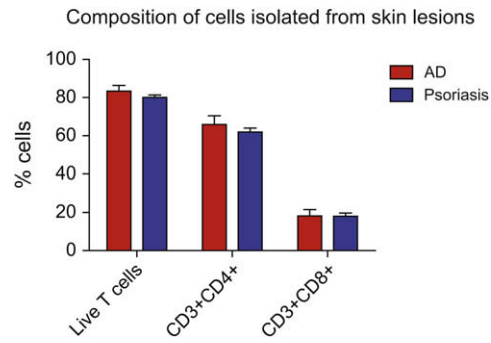


FIG E1. Similar composition of cells isolated from AD and psoriasis lesions. Percentages of isolated T cells, CD4⁺ T_H cells, and CD8⁺ T-cytotoxic cells are statistically similar between AD and psoriasis skin lesions.

TABLE E1. Antibodies used in flow cytometry

Antigen-fluorochrome	Manufacturer	Clone	Isotype	Dilution
CD3–Pacific Blue	eBioscience (San Diego, Calif)	OKT3	IgG _{2a}	1:40
CD4-phycoerythrin-Cy7	eBioscience	RPA-T4	IgG ₁	1:200
CD8-PerCP-Cy5.5	BD Pharmingen (San Jose, Calif)	SK1	IgG ₁	1:200
IL-17 Alexa 488	eBioscience	eBio64 DEC17	IgG ₁	1:20
IL-4-phycoerythrin	BD Pharmingen	3010.211	IgG ₁	1:20
IL-22-allophycocyanin	R&D (Minneapolis, Minn)	142928	IgG ₁	1:20
IFN-γ–Alexa 700	BD Pharmingen	B27	IgG ₁	1:500